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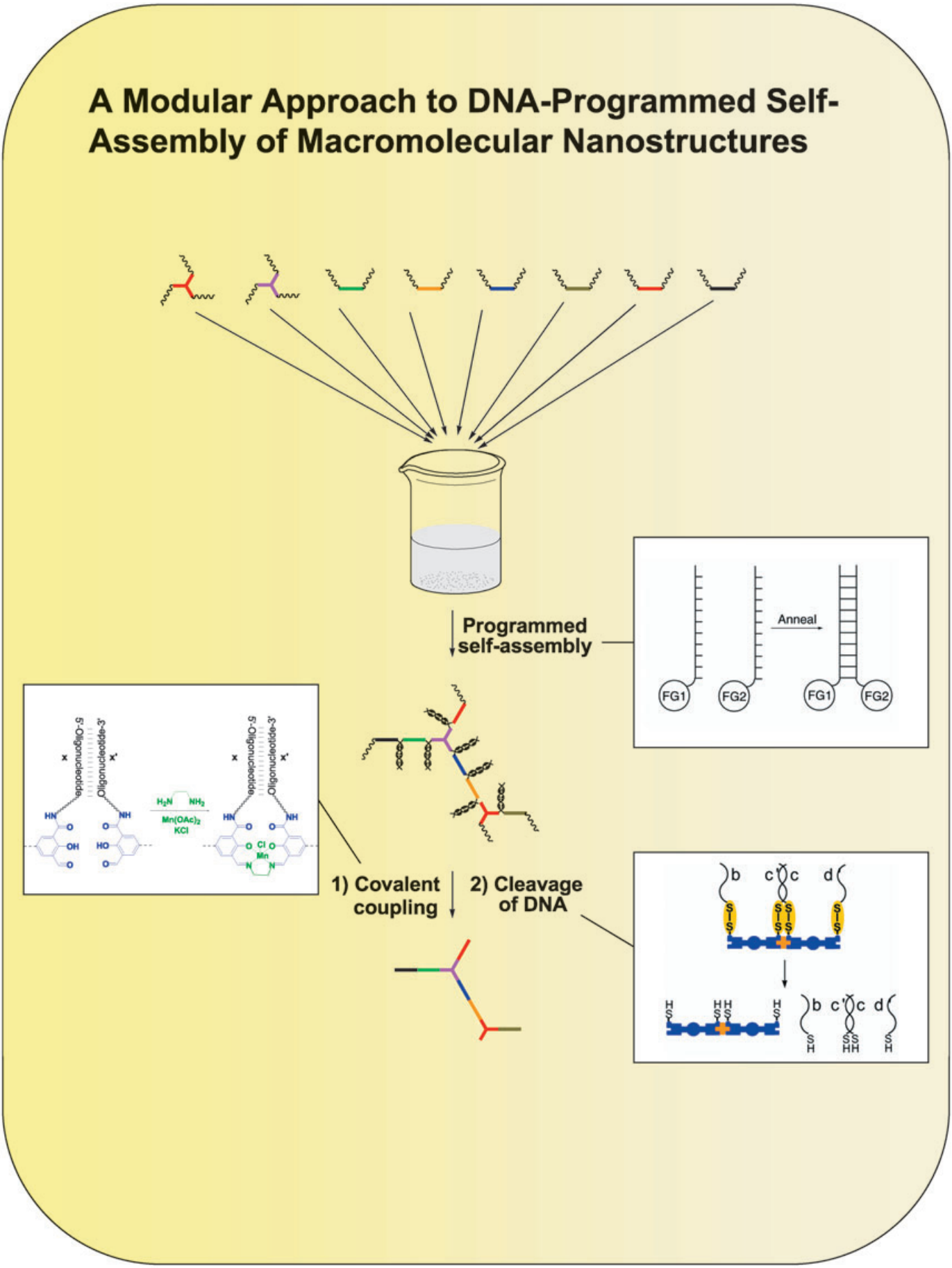

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A Modular Approach to DNA-Programmed Self-Assembly of Macromolecular Nanostructures

Kurt V. Gothelf*[a] and Raymond S. Brown[b]

Abstract: DNA-programmed organic reactions are new and powerful tools for assembling chemical compounds into predetermined complex structures and a brief review of their use is given. This approach is particular efficient for the selection and covalent coupling of multiple components. DNA-templated synthesis is used for polymerization of PNA tetramers and for copying of the connectivity information in DNA. Direct DNA-programmed multicomponent coupling of custom designed organic modules is described. The macromolecular structures obtained are highly conjugated potentially conducting nanoscaffolds. Some future developments in this area are discussed.

Keywords: DNA structures · nanostructures · oligonucleotides · self-assembly · template synthesis

Introduction

The major challenge for chemical nanotechnology is to develop simple and effective methods to transform molecular building blocks into complex nanostructures. Nature is extremely efficient in constructing complex functional macromolecular structures. This is achieved by the extraordinary ability to create a huge variety of oligomers, such as proteins, from a small set of 20 amino acids. The most significant difference between the chemical reactions, which are occurring in living organisms, and those performed in a laboratory, is the presence or absence of other reactive compounds. Chemical reactions in Nature take place in aqueous media containing a wide variety of small and large organic compounds. In living cells, the high specificity and substrate dependence in the formation of covalent bonds is accomplished by enzymatic processes developed over millions of years through evolution. In the chemical laboratory the products of organic reactions depend largely on the reactivities of participating compounds and functional groups.

This situation is changing rapidly through the emergence of Chemical Biology. In the last few years the field of DNA-directed chemical reactions has quickly developed.[1,2] This new chemistry has proved to be extremely useful for directing organic reactions in multistep synthesis,[3–5] asymmetric reactions,[6] DNA replication,[7] PNA synthesis,[8] assembly of nanostructures[9–11] and for combinatorial synthesis.[12] The basic principle of DNA-programmed synthesis is illustrated in Figure 1a.[1,2] The functional groups FG1 and FG2 can in principle react with each other without hybridization of the DNA sequences. However, they are very dilute and their intermolecular reaction is so slow that practically no conversion occurs by this route. If the attached DNA sequences are complementary, they will hybridize by base pairing to form double-stranded DNA even at very low concentrations. Annealing of the DNA sequences brings the two functional groups FG1 and FG2 in close proximity and they can react in a pseudo-intramolecular fashion, which proceeds significantly faster than the intermolecular reaction. Two other approaches to DNA-directed reactions are based on using either DNA hairpins or a DNA template (Figure 1b and c). More complex DNA structures for enabling DNA-directed reactions can also be envisioned.[5]

In earlier studies, DNA-directed reactions were limited to chemical ligation of oligonucleotides and their analogues.[1,2,13–17] The generality of this method has more recently been demonstrated by Liu and co-workers.[18,19] They

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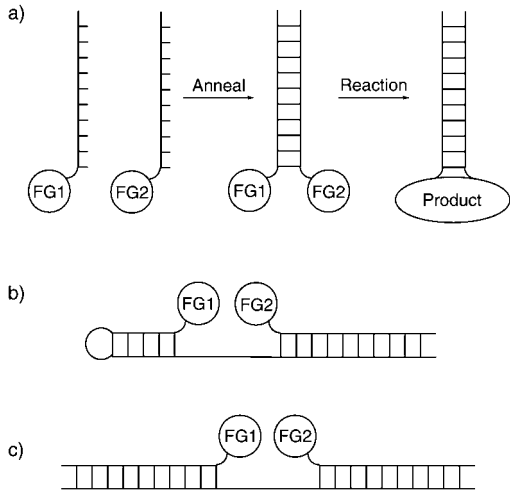

Figure 1. The principles of DNA-directed coupling reactions. a) Two functional groups FG1 and FG2 are brought in close proximity by annealing of their complementary DNA sequences. The pseudo-intramolecular coupling proceeds much faster than the non-directed intermolecular reaction. b) Arrangement of the reactants in a DNA hairpin structure. c) Localization of the reactants on a DNA template.

have reported a series of DNA-directed chemical reactions including, reductive amination, amide formation, S_N2 reactions, conjugate addition of thiols, amines and nitronates, nitroaldol condensation, Wittig reactions, 1,3-dipolar cycloaddition reactions of nitrones, and certain Heck reactions. A similar directed reaction, DNA-templated catalytic ester hydrolysis, holds great promise for application in drug release systems.[20–22] Metal–salen formation has also been demonstrated,[23,24] and used as a key reaction in the DNA-directed assembly of nanostructures (see below).[9–11] A highly attractive feature is that several otherwise incompatible functional groups can be present together but made to react specifically in the same reaction mixture.[25]

DNA hybridization, without formation of covalent bonds, has been exploited in several cases for the parallel assembly of multiple building blocks into supramolecular nanostructures.[26–29] The building blocks for such materials can be made of DNA alone. Seeman et al. have built elegant branched structures, such as, a DNA cube and even more complex geometric designs have been constructed.[30–33] Extension of this work applying DNA tiles was made by Seeman et al. and LaBean et al.[34–37] They were able to form and obtain images of highly regular two-dimensional DNA networks on surfaces by atomic force microscopy. DNA–organic hybrids consisting of a bent organic backbone attached to two complementary DNA sequences were assembled to form polydisperse cyclic structures.[38] Bunz et al. demonstrated the formation of linear assemblies of organometallic compounds attached to two different DNA sequences at the termini.[39] Stewart and McLaughlin made DNA–metal–organic hybrids with four DNA chains attached to a central organometallic core.[40] These structures were used to form branched supramolecular assemblies in parallel. DNA hybridization has also been applied to the assembly of conjugates with metal nanoparticles[26,27,37,41–43] and conjugates with proteins.[44]

This article deals with processes, which include both DNA-programmed assembly *and* formation of covalent bonds between multiple building blocks to generate macromolecular nanostructures. The contributions to this field from other groups will be described, but the main emphasis will be on our modular approach to the challenge of self-assembly and the concepts behind our work.

Templated Multicomponent Reactions

Non-enzymatic oligomerization of DNA- or RNA-based monomers by using an oligonucleotide template has attained significant success.[13,45,46] Despite important advances the sequence-specific copying of the template by parallel coupling of monomers has not yet been achieved. An efficient and sequence-specific translation of up to 40-nt DNA sequences into peptide nucleic acid (PNA) oligomers was recently reported by Liu et al.[8] The tetrameric PNA aldehydes were used as the building block for polymerization (Figure 2a). The DNA template aligns the PNA tetramers for coupling by reductive amination. Previous attempts to oligomerize PNA bases by acylation reactions gave limited regioselectivity and low yields. Unlike the acylation reaction, reductive

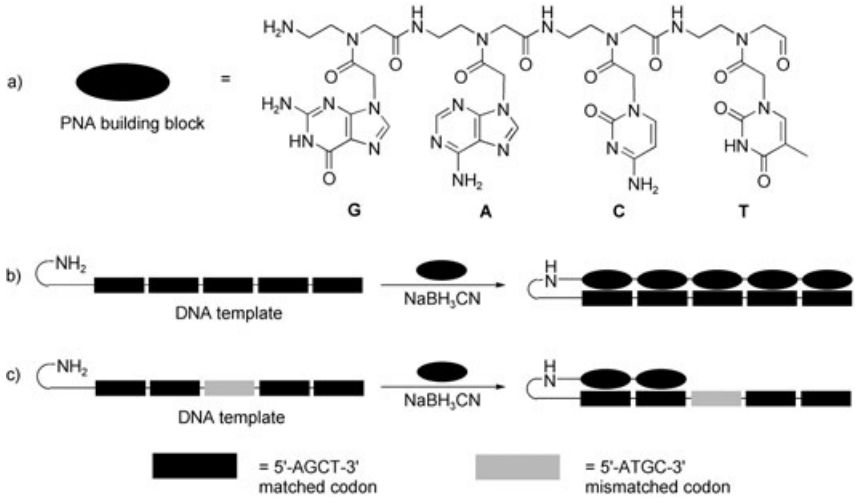

Figure 2. Translation of DNA into PNA polymers. a) Structure of the PNA building block. b) Templated polymerization of five PNA tetramers. c) Polymerization using a template with a mismatch in the third codon leading to coupling of only two PNA tetramers.

amination is highly distance dependent and the templated polymerization, shown in Figure 2b, proceeds in >90% yields. The 5′-amino terminal DNA hairpin template is linked to the PNA product in the coupling reaction. The sequence specificity and regioselectivity was tested by building a mismatched 4-nt codon with one or two mismatched bases (Figure 2c) into the template. This results in premature termination of the oligomer before the mismatched codon. A mismatched codon can be build into any position of the template and always results in termination of the growth of the PNA polymer in the amino-terminal direction.

Assembly and covalent coupling of three different oligonucleotides to a central organic core was reported by Kiedrowski and co-workers.[7] They used a three-armed DNA structure as the template to assemble the components for the chemical reaction (Figure 3). The three oligonucleotides each have a hydrazide moiety in their 5′-termini, which can react with aldehydes. Hybridization with the DNA template arranges the three hydrazide–oligonucleotides favorably for reaction with 1,3,5-triformylbenzene by tris-hydrazone formation. The hydrazone is reduced when the reaction is carried out in the presence of $NaBH_3CN$ as shown in Figure 3. Release of the reaction products under denaturing conditions reveals formation of a "negative" copy of the original DNA template. In this method the chemical connectivity information contained in the DNA sequences is copied by template-directed linking, and is an important step towards replication of DNA nanostructures.

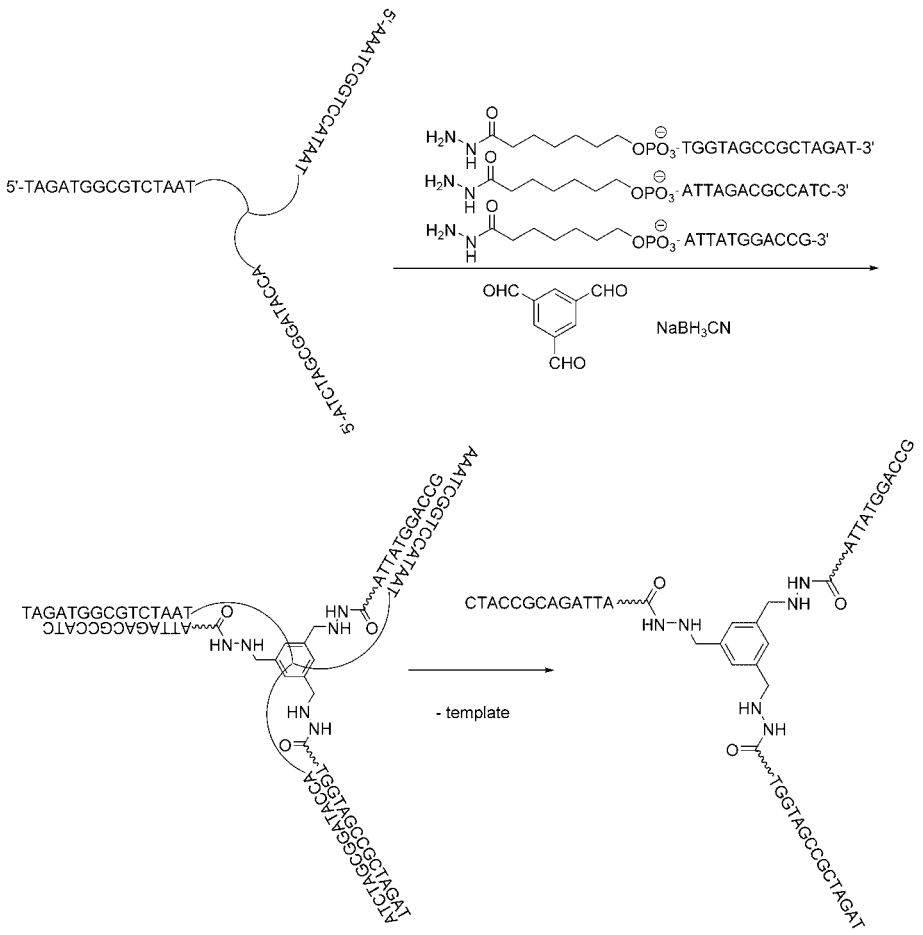

Figure 3. Copying of chemical connectivity information in DNA sequences by using a three-armed template.

Direct Multicomponent Couplings

The construction of new nanodevices that are non-symmetric and complex in design requires precise control over each new connection made when building the structure. In this regard conventional polymer chemistry and supramolecular chemistry are not useful since these are mainly limited to the production of polydisperse structures or a single highly symmetric monodisperse structure. Conventional total synthesis is the best way to make small or medium-sized molecules. But making huge complex molecular structures by traditional chemistry is very time consuming and extremely expensive. Thus the major obstacle to success is the need to develop a suitable molecular material with built-in coding features and the ability to assemble into the desired nanostructures.

In our attempt to find a solution to this basic problem, DNA-programmed reactions are used to arrange a set of basic molecular building blocks with the desired functionality for coupling in a predetermined specific manner.[9] The strategy outlined in Figure 4, does not require a DNA template. Each organic module has two or three built-in DNA sequences, which contain the information that governs the template-less assembly of multiple components. Each terminus also has a functional group, which can react covalently with other modules in the DNA-programmed reaction. In the absence of DNA, and at higher concentrations, the functional groups can react and eventually lead to polymerization. In contrast, in the DNA-programmed approach, reaction takes place only when functional groups are clamped together by the hybridized DNA sequences in a unique arrangement. In this way a predetermined nanostructure is formed by multiple parallel DNA-directed couplings. Subsequently it is desirable, for example, for scanning probe microscopy studies, that the DNA sequences are cleaved off and removed.

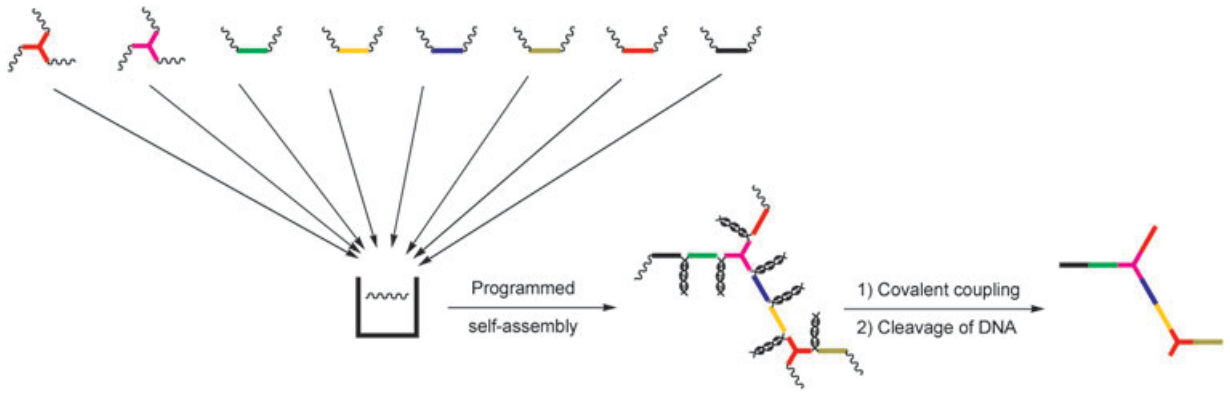

Figure 4. Principle of template-less DNA-programmed coupling of multiple modules.

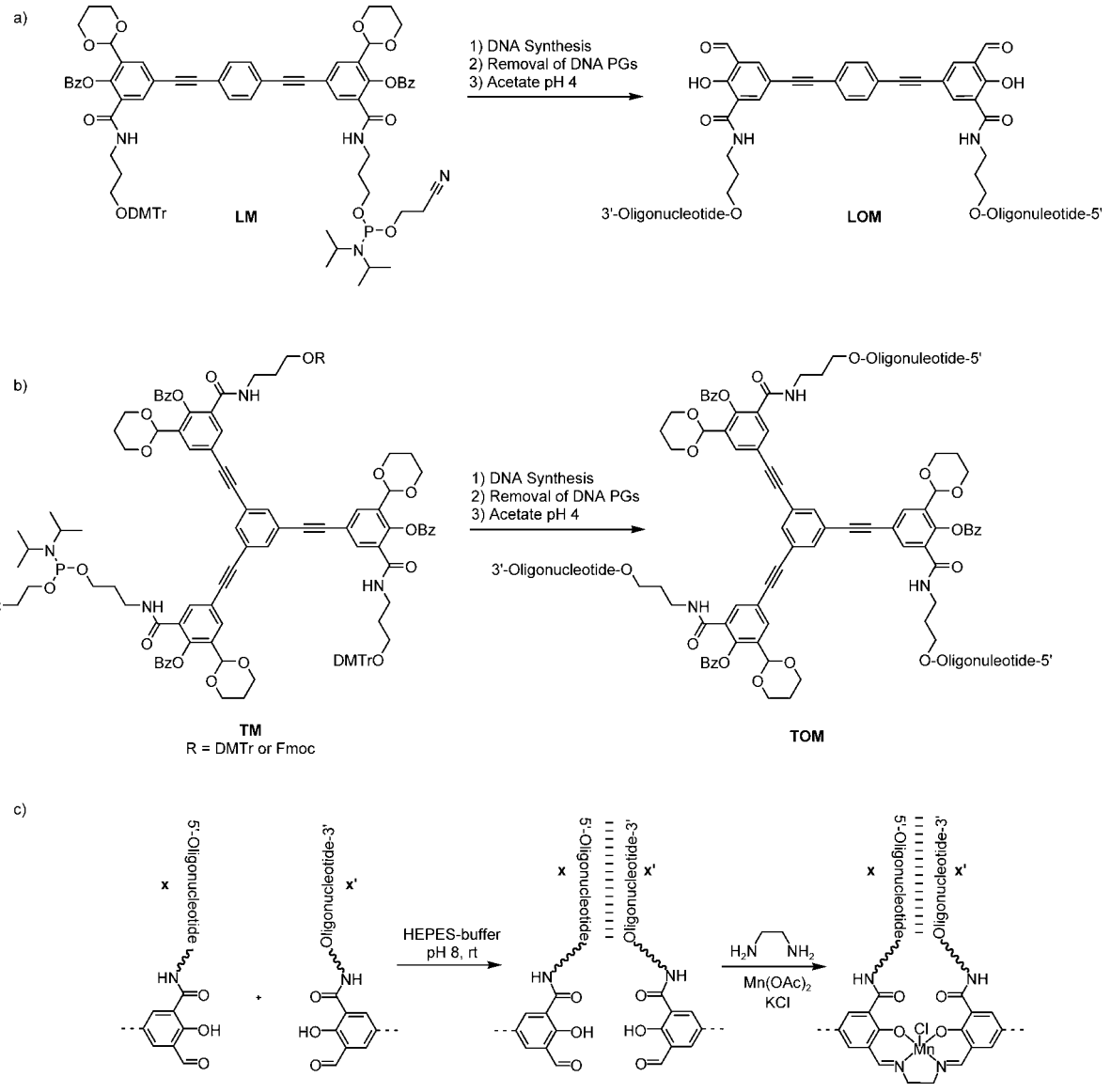

Figure 5. Formation of a) LOMs and b) TOMs from their organic precursors by automated oligonucleotide synthesis. c) DNA-directed manganese-salen formation.

To realize this concept we have designed and synthesized a linear module (LM) and a tripoidal module (TM) as shown in Figure 5.[11] The backbone of the modules is based on oligo-
(phenylene ethynylene)s to obtain a rigid, conjugated and potentially conducting structure. Oligo(phenylene ethynylene)s have previously been extensively studied and utilized as molecular wires.[47,48] In addition, the modules have protected salicylaldehyde moieties at each terminus. The salicylaldehyde groups are protected because they are incompatible with oligonucleotide synthesis.[23] The LM and TM also contain amide spacers at each terminus, which will function as connectors to the oligonucleotide chains. All of the terminal hydroxy groups of the spacer are functionalized with phosphoramidite, DMTr and Fmoc moieties for application of the module as a pseudo-nucleotide in automated DNA synthesis. Via this approach the LM and TMs were built into DNA sequences by phosphoramidite-based oligonucleotide synthesis to give the linear oligonucleotide-functionalized module (LOM) and the tripoidal oligonucleotide-functionalized module (TOM). The benzoyl and acetal protecting groups were subsequently removed under mild conditions.

Coupling of the modules exploits metal–salen formation as the key reaction for building up multicomponent molecular structures as shown in Figure 5c.[9] The salicylaldehyde groups of two modules are brought in close proximity when their complementary DNA sequences are annealed together. This enables us to control coupling of the functional groups to give a manganese–salen link between two modules by reaction with ethylenediamine and a manganese salt. Since this is a pseudo-intramolecular reaction it is considerably faster than the non-directed reaction. No additional DNA template is needed since the oligonucleotides attached on either side of the salicylaldehyde groups act as clamps to hold the organic modules in a predetermined arrangement. There are several reasons for choosing metal–salen formation as the key reaction for producing the links between the individual modules. The manganese–salen formation is a well-established reaction, which proceeds efficiently in aqueous solution.[23] The salen ligand adopts a locked coplanar geometry by chelating a manganese ion. Substituents attached to the 5-positions of the two salicylaldehydes are oriented in an essentially linear fashion. The metal–salen link constitutes a potential conducting junction with the possibility of varying the central coordinated metal.

The two basic modules can thus be encoded, as described above, to give a whole arsenal of LOMs and TOMs with different DNA sequences. Combinations of LOMs and TOMs with the desired encoding enables self-assembly and covalent coupling into predetermined nanostructures as depicted schematically in Figure 6. The figure shows a selection of different structures that were synthesized by self-assembly and coupling of the modules.[9] Couplings involving only LOMs result in a linear structure (Figure 6a), whereas the application of TOMs extends this concept into two dimensions. The coupling reactions proceed in parallel, and give

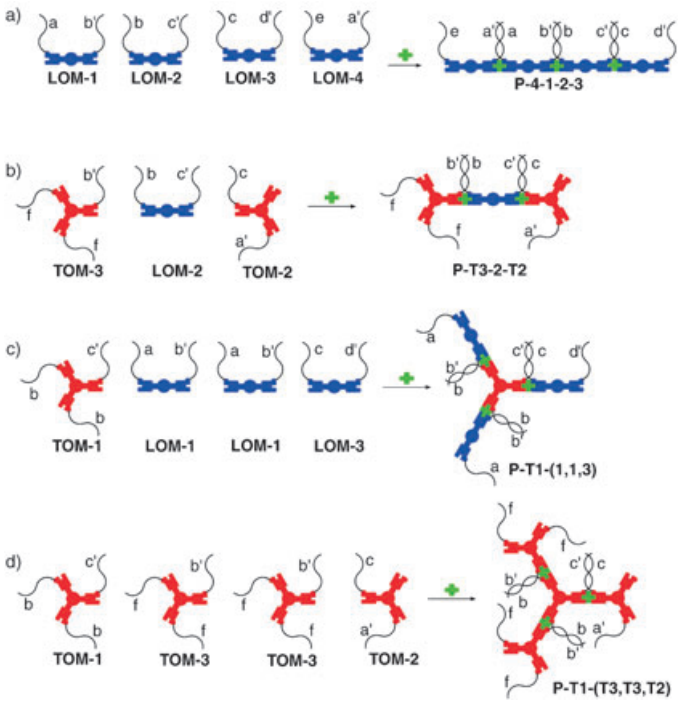

Figure 6. Representative couplings of LOMs and TOMs performed by formation of multiple manganese–salen complexes between the modules. Color code, blue: LOM, red: TOM, green: coupling reagents ($Mn(OAc)_2$, ethylenediamine). The size of the oligonucleotides (black curved lines) has been reduced compared to the actual proportions for clarity.

the macromolecular products in good to excellent yields, as estimated by denaturing polyacrylamide gel electrophoresis (PAGE). For example, the tetra-TOM product **P-T1-(T3,T3,T2)** containing 165-nt is obtained in more than 90% conversion.[9] A series of control reactions have shown that, in the absence of complementary sequences, or in the absence of ethylenediamine or $Mn(OAc)_2$, only unreacted monomers are observed by denaturing PAGE. Dimer and trimer products were characterized by MALDI-TOF MS and it was found that the melting points of the LOM/LOM, LOM/TOM or TOM/TOM combinations are increased by 15–30 °C after coupling of the modules.[9]

DNA-directed coupling of non-complementary modules can be accomplished by the application of an additional DNA template bridging the pair of oligonucleotides. This is shown for the coupling of two LOMs in Figure 7.[49] The DNA template contains 15-nt complementary to **b′** in **LOM-1** and 15-nt complementary to **c** in **LOM-3**. Spacing between the **b** and **c′** sequences in the template is not critical for the reaction to proceed. DNA templates having from one to four additional thymidines in between the **b** and **c′** sequences gave similar results. This is an important extension of our concept as it expands the diversity of assemblies that can be made from encoded LOMs and TOMs by the simple addition of a chosen DNA template.

The coupling of encoded modules into defined nanostructures is not limited to parallel assembly.[49] Figure 8 shows

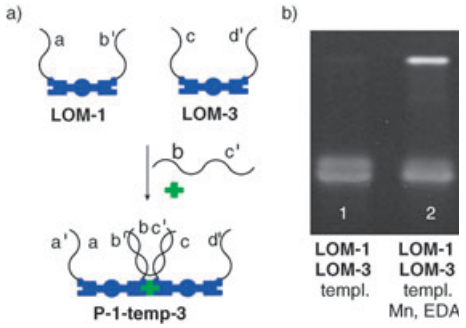

Figure 7. a) Templated coupling of LOMs and b) denaturing PAGE. Lane 1, annealing of LOMs and the template in the absence of coupling reagents. Lane 2, annealing of LOMs and the template in the presence of $Mn(OAc)_2$ and ethylenediamine.

Figure 9. DNA-programmed coupling of LOSM modules by aluminium-salen formation and subsequent release of DNA by reduction with TCEP. The coupling reagents $Al(NO_3)_3$ and ethylenediamine are shown in orange.

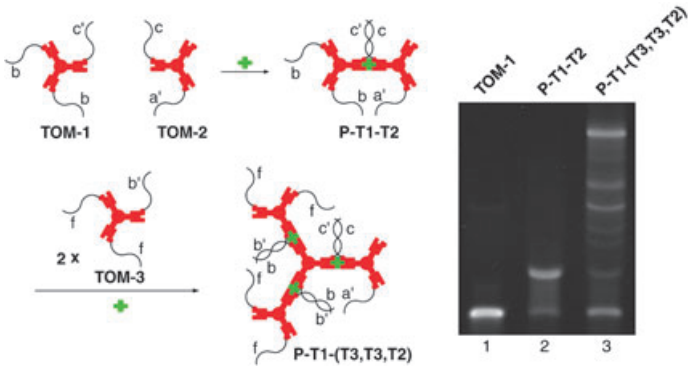

Figure 8. Serial coupling of TOMs. Denaturing PAGE analysis: lane 1, **TOM-1** (45-nt); lane 2, **P-T1-T2** (75-nt); lane 3, **P-T1-(T3,T3,T2)** (165-nt).

how the modules can also be used in a serial assembly of organic nanostructures. The dimer **P-T1-T2** can be formed in high yield and after its formation it can be linked in a second step two equivalents of **TOM-3** to form the tetramer **P-T1-(T3,T3,T2)**. The second coupling step is performed simply by addition of **TOM-3** to the reaction mixture. This demonstrates that the free salicylaldehyde functional groups of the modules are not blocked in the first step and can be used in subsequent coupling reactions.

For future applications and studies of the properties of these nanostructures it is desirable to remove the short DNA chains attached to each organic module. Introduction of a cleavable linker into the module further extends the design possibilities by enabling the removal of the DNA from an assembled nanostructure. A chemically reducible disulfide linker was introduced between the organic module and its oligonucleotide chains (Figure 9).[10] In this new LOSM (linear oligonucleotide functionalized disulfide module) species the DNA can be released by treatment with TCEP (triscarboxyethylphosphine) under mild conditions. DNA-directed coupling by aluminium–salen formation can be performed efficiently with LOSM modules. After reduction, the newly exposed organic backbone sulphydryls may be used for attachment to a surface, connecting to a nanodevice, or for the introduction of more functional groups into the structure.

The LOM and TOM modules described above are molecular building blocks, which can be combined in our bottom up strategy to form a variety of predetermined structures. DNA can be applied to direct the assembly and covalent coupling of multiple organic compounds in parallel with total specificity. Four modules can be coupled in high yields, whereas, pentamers and higher order LOM structures form in lower yields. We believe that it is most likely an adverse steric effect from sub-optimal packing of the DNA double helices together. Modification of the organic backbone or sequential release of the DNA from LOSMs may facilitate the parallel coupling of much higher numbers of modules.

Summary and Outlook

DNA-directed organic reactions undoubtedly have a promising future and several applications have been successfully demonstrated. The low atom economy of DNA-programmed synthesis imposes some limitations on the method, since many different oligonucleotides are needed, which are relatively expensive to obtain in large amounts. The power of the concept is the unique specificity, control and variety of transformations, which can only be performed by DNA-programmed synthesis. For analytical purposes, even minute amounts can be identified, since amplification of the DNA by means of PCR and sequencing can reveal the origin and/or identity of the organic reaction product. The concept can be applied widely and otherwise incompatible reactions can be carried out in the same mixture. This feature holds great potential for extending the chemistry from in vitro experiments to performing encoded reactions in vivo. This would require the use of stable DNA analogues, which are resistant to DNAses in living cells.

The main focus of this article has been the coupling of multiple encoded components, which may be the key to solving one of the greatest challenges of nanotechnology:

How to make molecules and/or materials capable of self-assembly into predetermined and stable structures, and extend the limits that can be reached by conventional synthesis.

The modular approach using encoded LOMs, TOMs and LOSMs developed in our laboratories is a proof of principle for a new bottom up chemical strategy. It is obvious that this concept can be extended to self-assembly of other organic compounds and perhaps to materials such as carbon nanotubes. The two-dimensional DNA networks on surfaces, as reported by LaBean et al.,[35] may also serve as a template for DNA-directed couplings to construct very large macromolecular structures. Integration of single molecules into molecular circuits is a basic unsolved problem in the emerging field of nanoelectronics. Since both the conjugated organic backbones and the metal–salen linkages between the modules are potential conductors, our method provides an important step towards the solution of this problem.

In the long term, the DNA-programmed approach may be the key to create smart functional nanodevices, often referred to as nanorobots. It is envisioned that this technology could be used to build predesigned macromolecular machines, which can perform specific chemical or biochemical reactions, release drugs or produce electrical signals.

Acknowledgement

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