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Self-assembly of aluminium–salen coupled nanostructures from encoded modules with cleavable disulfide DNA-linkers†

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DNA-directed coupling of organic modules by formation of stable aluminium–salen complexes, makes possible the subsequent reductive cleavage of disulfide linkers and release of the two oligonucleotide chains attached to each building-block.

The design and control of organic reactions using DNA-templates to select and precisely position molecules is a new and exciting area. Studies by Liu *et al.*[1] and others[2,3] have shown the versatility of these new and highly selective reactions for combinatorial chemistry, DNA-replication, asymmetric reactions and PNA-synthesis.

We have recently reported a templateless strategy for self-assembly and covalent linking of encoded molecular modules into nanostructures with a predetermined connectivity.[4] The method uses short attached complementary DNA-sequences to control the assembly of multiple modules. The resulting structures consist of a network of linear and branched oligo(phenylacetylene)s which are linked by conjugated and rigid manganese–salen complexes and these nanoscaffolds are potential conductors for nanocircuits.

For future applications and studies of these macromolecular nanostructures it is required to develop a method by which the oligonucleotides attached to each organic module can be removed. Introduction of a cleavable linker into the module further extends the design possibilities by enabling the selective or total exclusion of the DNA from an assembled nanostructure. Attempts to degrade the oligonucleotides completely by DNAse digestion were unsuccessful. Problems arise because both single and double-stranded DNA components require enzymatic digestion and end products are usually dinucleotides which will remain attached to the modules. We have, therefore, explored the introduction of a chemically reducible disulfide linker between the organic module and its attached DNA chains.

The linear module (LM)[4,5] was used for the synthesis of the linear oligonucleotide-functionalised disulfide-linked module (LOSM) as illustrated in Fig. 1. LM was obtained by a series of synthetic steps starting from a 5-iodosalicylic acid as recently reported.[5] The LM was incorporated by automated oligonucleotide synthesis using 5′-DMTr protected 3′-phosphoramidite nucleosides. Initially a chain of 15 conventional DNA bases was synthesized. Then the disulfide spacer followed by LM and another disulfide spacer were attached to the oligomer. Finally, a sequence of 15 conventional nucleotides was added to the chain in an iterative manner. To avoid unwanted side reactions *tert*-butylphenoxyacetyl protecting groups were applied to the nucleoside bases. This allows deprotection of the bases by treatment with 25% aqueous ammonia at 50 °C with no effect on the peptide bond of the LM. During this process the benzoyl groups of the phenols in the organic module are removed. Subsequently, aldehyde protection groups are removed by treating the acetal with an acetate buffer at pH 4.[2] The LOSM modules obtained in this manner couple with the same efficiency as the previously reported LOMs (linear oligonucleotide-functionalised modules).†[4] This is remarkable when compared to the LOM dimerization, since the double stranded DNA clamp directing the reaction is now located 17 bonds further away from the reacting center in the LOSM dimerization. Similar independent-of-distance results were also reported by Liu and coworkers for other DNA-directed coupling reactions.[1]

The manganese–salen-coupled organic backbone was found to be chemically unstable in the presence of reducing agents. When the redox sensitive Mn(III)–salen coupled LOM dimers were treated with 2-mercaptoethanol, dithiothreitol or tris(2-carboxyethyl)phosphine (TCEP) they decomposed into monomers (data not shown).[6] After testing a series of different metals in the salen forming coupling reaction it was found that aluminium–salen-coupled LOM dimers are stable and are unaffected by treatment with TCEP.

We have developed the DNA-directed formation of aluminium–salen complexes between two salicylaldehydes as illustrated in Fig. 2A. The salicylaldehyde groups are clamped together by hybridisation of the two attached 15-nt oligonucleotides with com-

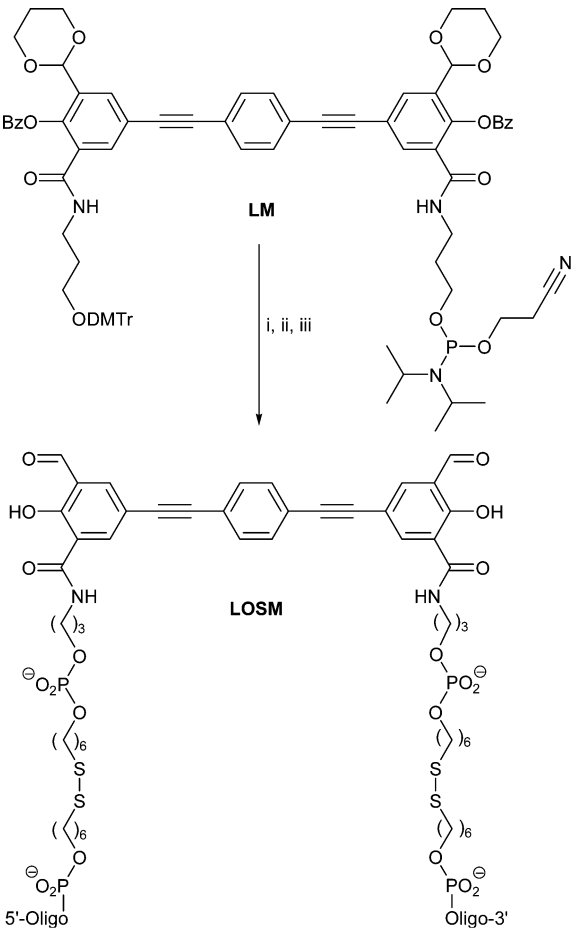

Fig. 1 Formation of linear oligonucleotide-disulfide-functionalised module (LOSM) from the linear module (LM). *i* automated oligonucleotide synthesis using phosphoramidite nucleosides. *ii* 25% aqueous ammonia 50 °C *iii* acetate buffer at pH 4, 37 °C, 2 h.

† Electronic supplementary information (ESI) available: experimental procedure, structures of LOM-**4** and LOM-**5**, native PAGE gel of the coupling reaction and MALDI-TOF characterization of LOSM-**1** and LOSM-**2**. See http://www.rsc.org/suppdata/cc/b4/b403956d/

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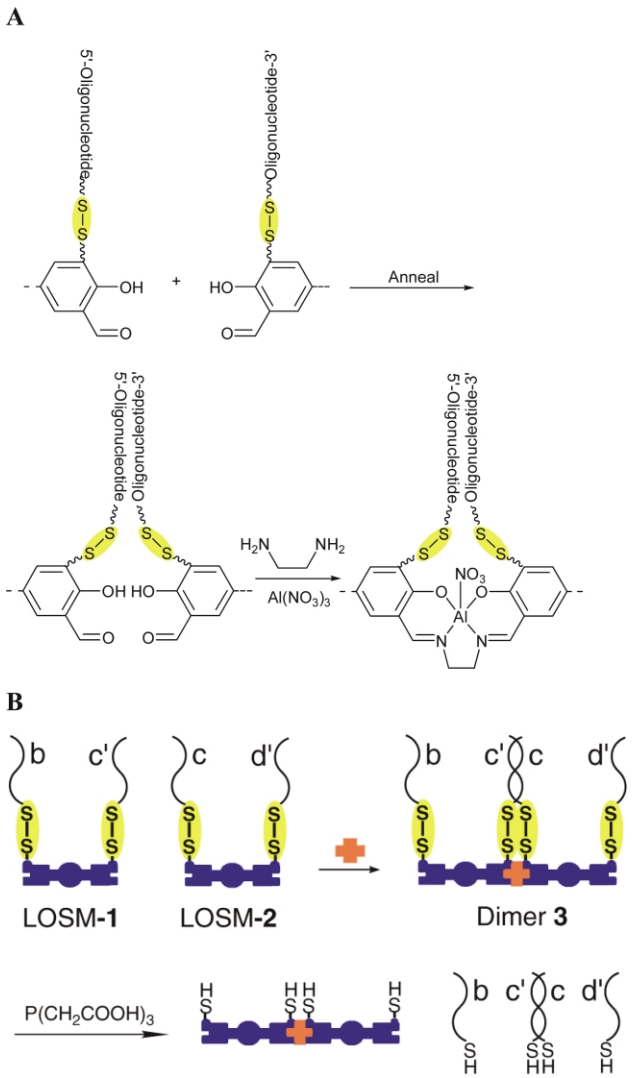

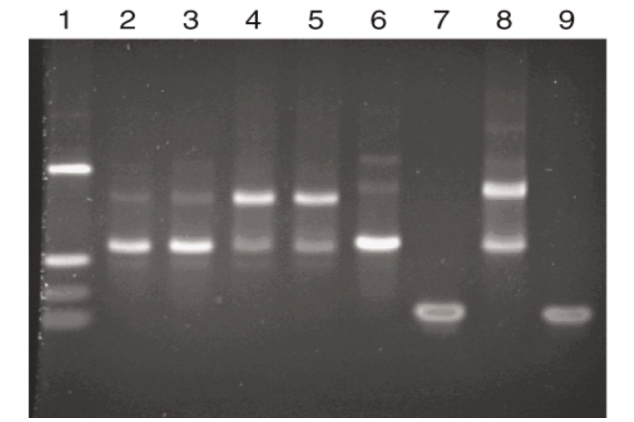

Fig. 2 Analysis of LOSM coupling and disulfide reduction with TCEP. **A**, DNA-directed aluminium–salen formation. **B**, Scheme of LOSM coupling reactions and reduction of Dimer **3** with TCEP, **b**: AAGTGTAGATGTACA, **c′**: ACTTCAGTTGGTCGT **c**: ACGACCAACTGAAGT, **d′**: CTGTAGACATATGTT. **C**, Electrophoresis in 8 M urea on a denaturing polyacrylamide gel stained with ethidium bromide and photographed in UV light. Lane 1: DNA markers 65, 31, 21 and 11 nt; lane 2: unreacted LOM-**4** and LOM-**5**; lane 3: LOM-**4** and LOM-**5** with TCEP; lane 4: Al^{3+}/EDA coupled dimer of LOM-**4** and LOM-**5**; lane 5: LOM-**4**-LOM-**5** Al-dimer with TCEP; lane 6: LOSM-**2**; lane 7: LOSM-**2** with TCEP; lane 8: Al^{3+}/EDA coupled Dimer **3**; lane 9: Dimer **3** with TCEP. The uppermost gel band is dimer (60 nt), middle band is monomer (30 nt) and the lower band contains reduced oligonucleotides (15 nt).

plementary sequences. Salen formation is greatly facilitated by the resulting close positioning of the salicylaldehydes.

The coupling of modules LOSM-**1** and LOSM-**2** were performed in the presence of aluminium nitrate and ethylenediamine (EDA) to give dimer-**3**. Subsequent cleavage of the disulfide bond and reaction products are described in Fig. 2B.‡ The aluminium–salen complex dimer–**3** was formed in more than 75% yield as estimated from the denaturing PAGE analysis of the reaction (Fig. 2C, lane 8). 8 M urea melts the DNA clamp but does not disturb the metal–salen link, allowing separation from the unreacted monomers. It was found that the dimer yield can be increased by replacing 0.5 M KCl with KNO_3 in the annealing and coupling reaction.

Mild conditions using 1 mM TCEP for 1 h at rt completely reduce the disulfide-linked oligonucleotides in contrast to dithiothreitol where a higher concentration of 50 mM is required. The disulfide linked oligonucleotides of LOSM-**2** were cleaved to yield free 15-nt chains (Fig. 2C, lanes 6–7). As expected when aluminium salen-coupled LOSM Dimer **3** is treated with TCEP, the DNA products released are two 15-nt oligonucleotides and a 15-bp duplex (Fig. 2C, lane 9).†

We have designed and synthesized self-assembling LOSM modules containing disulfide spacers between their organic moiety and their attached oligonucleotides. DNA-directed coupling by aluminium–salen formation can be performed efficiently with LOSM modules. More LOM or LOSM modules can be added to build molecular wires or combined with TOMs (tripoidal oligonucleotide-functionalized modules)[4] to construct stable 2-dimensional networks. The DNA chains required for self-assembly can be subsequently removed, if and when desired, by treatment with TCEP under mild conditions. Following reduction, the newly exposed organic backbone sulfhydryls may be used for attachment to a surface, linked to a nanodevice, or for the site-specific introduction of additional functional groups into the structure.

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Notes and references

‡ DNA sequences are: LOSM-**1**, 5′-AAGTGTAGATGTACA-SS-LM-SS-ACTTCAGTTGGTCGT-3′. LOSM-**2**, 5′-ACGACCAACTGAAGT-SS-LM-SS-CTGTAGACATATGTT-3′ (SS: disulfide spacer). LOM-**4** and LOM-**5** have identical DNA sequences to LOSM-**1** and LOSM-**2** respectively.

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