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Hetero-oligonucleotide nanoscale tiles capable of two-dimensional lattice formation as testbeds for a rapid, affordable purification methodology†

Philip S. Lukeman*

New nanoscale hetero-oligonucleotide tiles are assembled from DNA, RNA and morpholino oligos and purified using size exclusion filtration. Homologuonucleotide tiles assembled from RP-cartridge processed DNA oligos are purified by nondenaturing gel electrophoresis. These tiles’ purity and homogeneity are demonstrated by gel electrophoresis and their incorporation into two-dimensional arrays visualized by AFM. This purification methodology increases throughput and decreases costs for researchers who wish to screen multiple tiles for utilization in structural or analytical studies.

DNA-based nanotechnology (“DBN”) allows the construction of nanoscale objects, lattices and devices; however, DNA is not the only polymer that can take advantage of the specificity of the Watson–Crick base-pair to achieve these goals. Central to the implementation of DBN is the junction, a point where multiple helix axes converge. Previously, incorporation of L-DNA,3 methylphosphonate4 and GNA5 into single junction-based structures has been examined. Junctions are structural elements in double crossovers, multiply armed tiles and DNA origami; DNA double-crossover (“DX”) tile based arrays2 have been constructed using PNA6 and LNA7 oligonucleotides. RNA/DNA duplexes have been used8 for the assembly of multiply armed tiles8 and as a template9 to fold DNA origami;10 all-RNA systems known as ‘tecto-RNA’ have been used to generate a wide variety12,13 of structures.

With the exception of DNA origami and single-stranded tile systems, prototyping ‘tile’-based systems for incorporation into arrays has previously been conducted with (i) denaturing gel purification of constituent strands followed by (ii) the careful control of stoichiometry by titration or non-denaturing gel purification. This approach is time-consuming and costly.

Taking cues from the techniques developed for DNA origami assembly and purification,14,15 here we demonstrate a time and cost-saving methodology that overcomes the need for denaturing gel strand purification and careful stoichiometry control in the assembly of multi-stranded tiles and their incorporation into two-dimensional lattices.

We prototype this rapid and affordable tile purification methodology by using an existing array design and then, for the first time, incorporating RNA segments into DNA DX tiles capable of forming the AB* two-dimensional array9 (Fig. 1) in a manner analogous to previous studies.6,7 We test the effectiveness of these new tile’s assembly and their purity by (a) non-denaturing gel electrophoresis (Fig. 2) and (b) AFM analysis of two-dimensional array formation (Fig. 3). Successful array

Fig. 1 Strand diagram, array formation and tile nomenclature. (i) Strand diagram of the AB* system6 consisting of two DX tiles (A and B) designed to tile the plane via sticky-ended cohesion. (ii) Well-formed arrays are planar and have features (that result from the hairpins in the B tile) that are ~32 nm apart as shown schematically here. Helical regions are abstracted as cylinders, hairpins protruding from surface of array are abstracted as dots. Image reproduced with permission from ref. 7. (iii) A representation of the correction that the AB* system undergoes when B tiles that contain longer B-DNA outer arms are added to compensate for helical pitch differences in the red/green non-DNA polynucleotide duplex regions in the A tiles. (iv) This study describes synthesis of ‘A’ tiles that contain various non-DNA oligo-sections (RNA and/or morpholino) that affect the helical pitch of the red/green duplex region. The names of these modified tiles, and their corresponding changed oligo region are listed in the table.

† Electronic supplementary information (ESI) available: Experimental procedures, gel electrophoresis and AFM data. See DOI: 10.1039/c3nr01551c

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formation is predicated on good tile structure/homogeneity and co-planarity of tiles in the array; tile co-planarity is designed based on the helical repeat of duplex DNA. For non-DNA oligo-sections in the A tiles, we use B tiles with different arm lengths to compensate for helical twist change. B tiles with n extra base pairs in the outer arms compared to the one designed for all-DNA array formation are known as Bn tiles, so a B tile that has 1 extra base pair is called ‘B1’, 2 extra base pairs, ‘B2’ etc.

We negated the need for stoichiometry control in the assembly of the ‘A’ tiles using filtration through commercially available molecular size cutoff centrifugal filters. Fig. 2i shows a non-denaturing gel of the ‘A’ tile complexes used in this study where stoichiometry errors are revealed by lower molecular weight species in the gel; these lower molecular-weight species are removed by filtration. As a matter of course this quick process can be added to tile preparation as a standard post-annealing step of any new tile that is being investigated. This technique also minimizes-post assembly processing of nuclease-sensitive tiles that contain RNA. These ‘A’ tiles were used to form the arrays shown in Fig. 3 and in the ESI†

We negated the need for expensive and time-consuming multiple denaturing gel purification of individual DNA strands for ‘B’ tile assembly by using commercially available reverse-phase cartridge-processed strands followed by a single non-denaturing gel purification. Fig. 2ii shows a comparison of ‘B0’ tiles formed from denaturing gel-purified strands (B0gel) and the corresponding native-gel purified ‘B0’ tiles from significantly cheaper and quicker to obtain cartridge-processed strands (B0cart) – their homogeneity is comparable (see ESI† for tiles B0–B8 and an estimation of time saving and costs).

We then demonstrated that B0gel and B0cart gave comparable arrays when annealed with an all-DNA ‘A’ tile Add (Fig. 3i and ii). Having demonstrated that the rapid purification of the ‘all-DNA’ B0-Add pair allows array formation, we moved on to novel, RNA-containing A tiles. These A tiles were screened for array formation with cartridge-purified B tiles B0–B8; a suitably matched pair of A and B tiles is expected to give arrays. While RNA has been incorporated into multi-armed structures and origami previously, neither of these classes of structures utilized RNA in the ‘crossover’ strands; nor did they utilize RNA-RNA duplexes.

For array formation via the tecto-RNA approach, gel purification of individual strands and stoichiometry control was required.

The Ard and Adr tiles gave well-formed arrays with the B3 tile that showed regular features on the surface of arrays with the expected periodicity of ~33 nm (Fig. 3iii and iv). Annealing B2 with Adr/Ard formed ripped arrays; no arrays were formed with B4 and Adr/Ard.

The Arr tiles formed very large arrays with the B2 tile (Fig. 3v), but these were either ripped or ‘flat’, with no periodic features observed by AFM – we do not have an explanation for this effect – with Arr the B1 and B3 tiles did not form arrays (see ESI† for details of AFM experiments, Arm and Amr tile behavior and our confidence level in ‘no array formation’).

Unconstrained RNA-DNA and RNA-RNA junction behavior can be complex; however, the results above suggest that the DX motif held the junction in a somewhat antiparallel fashion as it does with other conformationally mobile junctions.
In conclusion, we have demonstrated rapid and cheap purification of homo- and new hetero-oligonucleotide tiles capable of forming DNA two-dimensional arrays. We expect these techniques will prove useful to researchers who wish to quickly screen multiple tiles for suitability of incorporation into arrays and other nanotechnological constructs.

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Notes and references