

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

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New nanoscale hetero-oligonucleotide tiles are assembled from DNA, RNA and morpholino oligos and purified using size exclusion filtration. Homo-oligonucleotide 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 nanotechnology¹ ("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,² methylphosphonate³ and GNA⁴ 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 arrays⁵ have been constructed using PNA⁶ and LNA⁷ oligonucleotides. RNA/DNA duplexes have been used⁸ for the assembly of multiply armed tiles⁹ and as a template¹⁰ to fold DNA origami;¹¹ all-RNA systems known as 'tecto-RNA' have been used to generate a wide variety^{12,13} 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 array⁵ (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

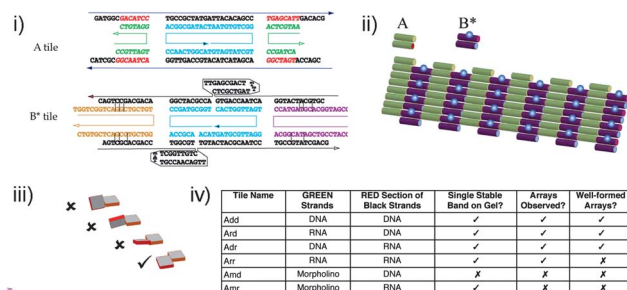


Fig. 1 Strand diagram, array formation and tile nomenclature. (i) Strand diagram of the AB* system⁵ consisting of two DX tiles (A and B) designed to tile the plane via sticky-ended cohesion. (ii) Well-formed arrays are planar and have features (that result from the hairpins in the B tile) that are ~32 nm apart as shown schematically here. Helical regions are abstracted as cylinders 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.

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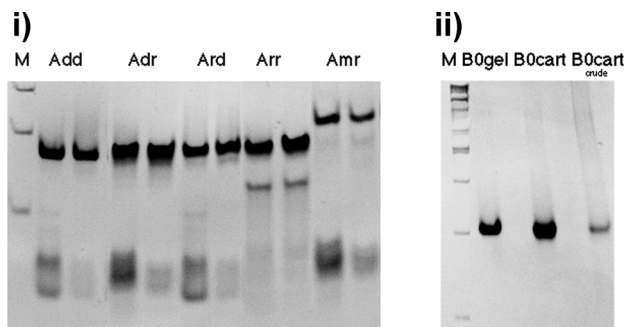


Fig. 2 Purification for rapid screening of oligonucleotide-based tile systems. i): nondenaturing gel showing the purification, using Microcon 30 kDa columns, of 'A' tiles that have stoichiometry errors. Equimolar quantities (10 pmol by A_{260}) of tiles Add, Adr, Ard, Arr and Amr are all shown, with the left lane showing the raw annealed sample and the right lane showing the sample after 4 passes through a column. Arrays formed with all but the Amr samples – the ESI† describes attempts to form arrays with the morpholino-containing tile Amr tile, experimental description and demonstration of the individual tiles' successful assembly compared with sub-tile components. ii): nondenaturing gel comparing tiles formed from denaturing gel-purified strands with native-gel-purified tiles formed from cartridge-processed strands. Equimolar quantities (10 pmol by A_{260}) are used. B0gel = B0 tile made from a stoichiometric anneal of equal quantities of strands that were denaturing gel purified. B0cart = B0 tile made from native gel purified complex made from cartridge-processed strands. B0cart_{crude} = B0 tile made from cartridge-processed strands. See ESI† for description/demonstration of the purification techniques in further detail and a cost/time analysis.

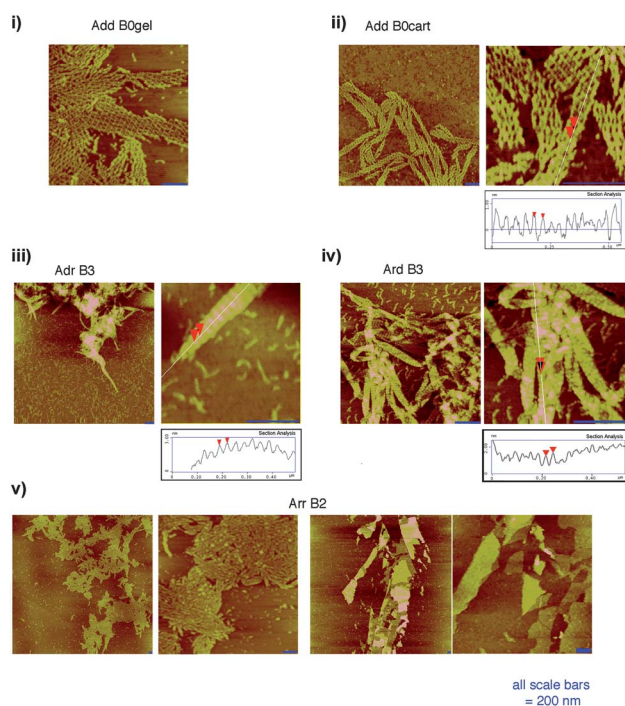


Fig. 3 Representative AFM images of AB* arrays. For (ii), (iii) and (iv), to the right of the main image is a higher resolution, 'zoomed' image of the same array and a height-cross-section showing the expected ~ 32 nm distance between array features. (i and ii) Comparison of B0gel and B0cart tiles with Add. Note arrays form well with either the expensive denaturing-gel purified B0gel or the cheap and quick purification method for B0cart. All arrays shown after this were formed with B tiles made from the quick purification method. (iii and iv) Adr and Ard tiles formed well-structured arrays with B3. (v) Arr formed large arrays ($> 5 \mu\text{m}$ width) of the expected height with B2. However, the arrays were either flat and featureless or ripped – no regular features were observed. See ESI† for other AFM data and experimental details.

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.⁶ 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' tile 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 structures⁸ and origami¹⁰ 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.^{12,13}

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, Arrm and Amr tile behavior and our confidence level in 'no array formation').

Unconstrained RNA–DNA and RNA–RNA junction behavior can be complex;¹⁶ 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.¹⁷



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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