Supporting Information

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

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Methods and techniques

Sources and purification of oligonucleotides.					
Oligo	Supplier/Purification	Notes			
DNA - cartridge DNA - other	Bioneer / RP cartridge IDT/ denaturing PAGE				
RNA RNA-DNA chimeras	IDT / HPLC	Aliquotted into 1 nmol samples, lyophilized and stored dry to minimize RNAse degradation. Fresh aliquot used for each expt.			
Morpholino	GeneTools / precipitated				

Sources and purification of oligonucleotides.

Unless stated otherwise, the samples were dissolved at 10 μ M (based on the quantities give by the suppliers) in water and stored frozen.

Water was purified through a Barnstead EasyPure II system to at least 18 M Ω . Unless stated otherwise, all reagents were from Fisher and used as received. All stock and buffer solutions were pre-filtered through a 0.2 μ m filter. A Hoefer SE600 gel system was used for all acrylamide gels.

Nondenaturing Polyacrylamide Gel electrophoresis. Gels contained 6-15% polyacrylamide (19:1 acrylamide/bisacrylamide) and were run at room temperature. The running buffer consisted of 40mM Tris, 40mM Acetic Acid (pH 8.0), 12.5 mM Magnesium Acetate, and 2 mM EDTA (TAEMg). Just prior to running, 10% v/v of a 50% glycerol / 50% aqueous TAEMg solution of 0.1% bromophenol blue and 0.1% xylene cyanol FF tracking dye was added to the sample. Gels were run on a Hoefer SE 600 electrophoresis unit (10 V/cm, constant voltage).

Visualization of gels was performed with SYBR Gold stain (Invitrogen) according to instructions from the manufacturer and imaged using a Gel Logic imaging system (Kodak), and the "invert" function used on Photoshop to invert the black/white scale.

Assembly of tiles. Strands were mixed in equal stoichiometric ratios at 1 μ m concentration (using quantities given by suppliers) in TAEMg and annealed from 90°C to 25°C over ~ 4h. For RNA-containing systems, the annealing temperature range was from 70°C to 25°C.

Assembly of AB* arrays: Tiles were mixed in a 1:1 stoichiometric ratio and annealed from 50°C to 25°C over 12-15h. Before annealing, some samples were supplemented with MgCl₂ to a final Mg²⁺ concentration of 100 mM. This affected array width but not formation.

Annealing/assembly was conducted in a 2L beaker filled with hot water with the samples in 0.5ml eppendorf tubes immersed beneath the liquid in a 50 mL centrifuge tube. For array anneals thermal equilibration was slowed down by insulating the beaker in a styrofoam box; this is a standard technique¹⁻³ that forms 2d arrays.

Purification of tiles from low MW contaminants by buffer exchange using Microcon Columns: 200-400 µL Samples were loaded into Microcon-30kdA units

(catalog # MRCF0R030) and exchanged with filtered TAEMg buffer 4 times according to the manufacturers instructions.

AFM Samples were deposited onto freshly cleaved mica for 2 - 60s, washed with ~1 mL of filtered water, wicked and dried with a stream of compressed air from a can. Samples were visualized using a DI Nanoscope IIIa AFM, J-Scanning head, tapping mode, in air, using PPP-NCH tips (NanoAndMore). These are techniques identical to those used in previous studies of AB* array systems with heterooligonucleotide tiles^{1,2}.

AFM Experiments: conclusiveness of 'arrays formed' vs 'no arrays formed'

We deposited samples in Mg²⁺-containing buffer for between 2-60s - in every sample we examined thoroughly, there was a considerable background of small random aggregates in every sample; experience has shown two-dimensional arrays and one dimensional systems adhere more strongly to the surface than small aggregates by virtue of their surface contact area with the mica.

We made sure that the AFM tip was arranged over the center of the mica where the deposition took place, and every sample was scanned for two separate 105 μ m fields at least 1mm apart in the center as below. At a minimum, the blue areas were scanned entirely

Looking for arrays: search was conducted as below.



With all of the systems that formed arrays, we observed some arrays within the first two 15x 15 μ m scans (225 μ m²). In the systems where we state no arrays formed, we observed no arrays in eighteen (15x15) μ m areas (4050 μ m²) spread out over > 1 mm. Given the prevalence of arrays found in earlier AB* systems, we believe our assertion of 'no array formation' is reasonable.

A tile strand nomenclature.

The 5' ends are labeled with the strand number.



The

Gels show the formation of the Axx tiles by the process of comparing the tile assembly with all the sub-complexes missing one strand. So for example, if the Ard tile contains strands 12345, the lane marked "-4" is the complex formed from strands 1235.

Gel data for complex formation and purification

Nondenaturing gel showing the formation of Adr and Ard.

While a stoichiometry error is clearly visible in the lower half of the gel (cleaned up in Fig 1 in main text via purification), the single main band has the expected mobility (+/- 5% with an authentic sample of Add tile).



Left to right: M, Ard, -1, -2, -3, -4, -5, M, Adr, -1, -2, -3, -4, -5

Nondenaturing gel showing the formation of Arr and Arm.

While a stoichiometry error is clearly visible in the lower half of the gel (cleaned up in Fig 1 in main text via purification), the single Arr band has expected mobility. (+/- 5% with an authentic sample of Add tile).

Notes: The Arr-2 lane is damaged, so this part of the gel is repeated below.

The Arm tile has a lower mobility than the other all-anionic tiles. This is as expected with a lower charge-to-mass ratio due to the incorporation of the two uncharged 15mer morpholino oligonucleotides.



Left to right: M, Arr, -1, -2, -3, -4, -5, gap, M, Arm, -1, -2, -3, -4, -5 **Fig 3a**: confirmation that Arr and Arr-2 have different mobilities.



Left to right: M, Arr, Arr-2

Demonstration of native gel purification of "crude" cartridge strands.

Sequences of tiles B0 to B8 were as described previously². Strands were obtained from Bioneer cartridge-purified at the 200nmol scale.

5 nmol of strands were mixed in a stoichiometric ratio according to the amounts described by Bioneer and annealed at 1 μ M in TAEMg. These are the 'crude' samples shown on left.

For purification, the samples were concentrated to ~10 μ M using a Microcon-10 concentrator** loading dye added and ~4 nanomoles were loaded onto a 3cm width lane of 1.5mm thick 8% acrylamide (19:1 Acrylamide:Bis) nondenaturing gel; the gel ran for 1200 Vh at room temperature and stained with ethidium bromide. The appropriate band was cut out, eluted in 2ml Elution buffer, extracted with butanol (3 x 500 μ L), precipitated by adding 4 5 volumes of EtOH at -40°C, the pellet washed with ice cold (-20°C) 80% ethanol, re-dissolved in TAEMg, filtered through a 0.2 μ m filter and then re-annealed at ~1.5 μ M concentration: the yield for this process was ~25% for each tile. These are the pure samples shown on the right of the gel.



Left->Right Crude B0, 1, 2, 3, 4, 5, 6, 7, 8, Marker, Pure B0, 1, 2, 3, 4, 5, 6, 7, 8

** Note;: the post-anneal concentration step is required so that the volume to load on the gel is not prohibitive; annealing these tiles at higher concentrations forms multimers⁵.

Cost/time estimates comparing purification methods

B0-B8 tiles: 30 unique strands, 9 tiles, ~2,000 bases.

Cost/time, based on list prices from bioneer (cartridge purified), or IDT (desalted), or IDT (gel purified). Regular purchasers would expect a 20-60% discount from these prices. Furthermore, ordering the oligos in 96-well plates saves an additional 30-60% on these values.

Cartridge strands: tile native gel purified. From start to finish 9 tiles could be obtained pure (from 3 gels) and stoichiometrically set in about 3 days. Strand cost from Bioneer for 30 cartridge purified strands \$940 at the 50 nmol scale or \$1800 at the 200nmol scale.

Crude strands : strands denaturing gel purified in-house. Comparison of time; to purify all tile strands by gel would require all 30 strands to be purified, ca. 10-15 gels, and associated workup. This would require ~8 days of work. Strand cost from IDT = \$975 at the 100nmol scale or \$1950 at the 250nmol scale.

Crude Strands gel-purified purified by supplier Strands ready for synthesis (100nmol) \$45/gel - extra cost \$1350 (250nmol) 69/gel . .

	This study	Usual technique ^{1, 2}	
	Cartridge/Native Pur	Crude/Denat Pur in-house	Crude/Denat Pur commercial
Cost	\$940	\$975	\$2325
Time	2-3 days	7-10 days	1-2 days

Supplementary AFM data

AFM Data indicates that for the Ard and Adr samples the B3 tile gave the best arrays: B4 gave no arrays and B2 gave 'torn' arrays on which the striping was not visible.

For the Arr tile, the B2 tile gave the best arrays, which while were either torn or featureless. Arr with B1 and B3 have no arrays, just random aggregates

Add B0cartridge

Add B0cart





Adr AFM

Fatten Fatten for the second second





Adr B2: note: arrays formed, but torn and features not as clear as B3

Arr AFM

Arr B2: supplemental images









Arr B3: note: no arrays, just aggregates.



Arr B1 : note: no arrays (flat round objects are some sort of evaporation phenomenon, not characteristic of arrays)





Arm with B0-8

Despite intensive screening, there were no arrays found with any of the combinations of Arm and B0-8; random aggregates and or objects <100nm² were seen. As stated in the text below, this is likely because of the heterogeneity of the strand as supplied: even if every Arm tile had full-length morpholino strands bound correctly, these morpholino strands could be one of thousands of diastereomers, each with its own helical pitch or distorted helix geometry.

Discussion on Morpholino tile formation and lack of arrays

Morpholino oligonucleosides are uncharged oligos whose ribose has been replaced by a morpholine, and the phosphate replaced with a neutral phosphorodiamidate⁶. These oligos are used extensively in knockdown studies and they pair in a discriminatory and mismatch-sensitive duplex manner with DNA⁷; their interaction with RNA, while specific, is more complex⁸. In both cases their duplex structural characteristics are unreported.

Attempts to use a DNA/morpholino duplex in assembly of a stable Amd tile failed (see below "Native Gel of Adm). Using a RNA/morpholino duplex in the analogous Amr tile generated a stable complex that, after quick purification, was homogenous by nondenaturing gel electrophoresis (see "Nondenaturing gel showing the formation of Arr and Arm). However, despite extensive testing against tiles B0-B8, no arrays were formed.

The morpholinos as supplied were heterogeneous both in strand length and in diastereopurity; purification of unfunctionalized morpholinos is challenging⁹. Given that a tight band with an 'A' tile's expected mobility forms on nondenaturing gels and the fact that using an increasing excess of morpholino does not affect this band's mobility, we believe (but cannot prove) that mostly full-length morpholinos form the Amr tile. There is a high mismatch penalty for sub-optimal binding to RNA, we use excess morpholino strand, which should allow the full-length strand to out-compete failure sequences as in DNA origami¹¹ and annealing over this time period should have allowed equilibrium to be reached in a system without kinetic traps¹¹.

In our opinion, the diastereoimpurity of the morpholino strands was the likely culprit for array failure in this case: as the phosphorodiamidate is chiral, and there is no control of chirality in the synthesis of the oligomer, each 15-mer morpholino strand could be one of 16,384 diastereomers, each with its own helical pitch or distorted helix geometry; we had hoped that these features would average out; we hypothesize that this was not the case.

Native gel of Adm

Showing annealed tile does not form one band even when single band is cut out and reannealed.



L->R Marker, "gel purified" and re-annealed Adm, gap, crude Adm.

We cut the major band out of a crude preparation of Adm (right band) and then re-annealed it. At equilibrium, we obtained a distribution of products indicating that this tile was not stable.

As RNA binding to morpholino oligonucleotides is stronger and more selective⁴ than to DNA, we hypothesized that the corresponding Arm tile would behave better on gel electrophoresis. This proved to be the case (see Fig 3).

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