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SUPRAMOLECULAR ASPECTS OF  
DNA-BASED ASSEMBLY

*Understanding the behaviour of complex, multi-component chemical systems is premised on embracing the full set of interactions that occur within the component molecules, between structures, and between the species and system at large. Broadly, these ideas can be discussed as elements of supramolecular chemistry and are well-illustrated in a diverse range of experiments related to DNA-based assembly systems. Through the study of DNA's role in mechanical templating, sequential selective base-pair bonding in constituent assembly, higher dimensional self-assembly, and mixed-constituent biased thermodynamic assembly, an understanding of supramolecular phenomena can be developed and appreciation for novel chemical pathways enhanced.*

DNA is remarkable not just for its behaviour in any given system but for the breadth of roles that it can hold. The iconic double-helices are primarily fascinating for their fundamental role in biological self-assembly but they are also exemplars in a set of unrelated regimes of organic and inorganic assembly as materials, templates, constituents, linking agents, labels in chemical addressing, and thermodynamic guides. More generally, DNA manifests an instructive blend of molecular and supramolecular behaviour that allows a diverse range of function in assembly systems. This paper will expound upon the latter supramolecular mode, elucidate the many ways in which DNA expresses these behaviours in assembly, and explore just how non-covalent bonding can get.

At its best and most basic, DNA can be described by the canonical Watson-Crick model, a double-helix of intertwined strands. The polynucleotide backbone of each strand is bound to its partner by a set of regularly-spaced bases attached up and down the spiraling strand. The backbone consists of alternating sugars and phosphates, and represents the rigidity of a given strand. The mechanical integrity of a single stand is thus conventional; properties are thoroughly the product of typical covalent bonding. It is through the attached bases, however, that novel behaviour emerges simply by virtue of their being defined by a limited number of special configurations. Not only are they not arbitrary, but they belong to a class of two base pairs (bp) that exhibit a specific affinity only for each other through hydrogen-bonding. It is

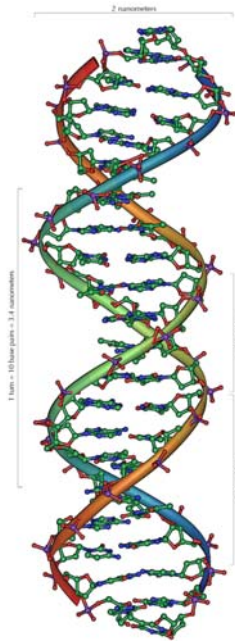


Figure 1 – Watson-Crick DNA

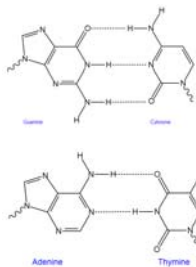


Figure 2 – GATC binding pairs

thus that non-covalent bonding becomes central to the DNA structure; the ensemble effect of a large number of relatively weak bonds acting complementarily is ultimately responsible for holding the structure intact in a flexible but topologically-sound geometry. Interestingly enough, the equilibrium result does not represent a symmetric form but rather one that is characterized by the winding of major and minor

grooves up the spine. In adenine (A) binding so well with thymine (T), and guanine (G) linking uniquely with cytosine (C), DNA has an inherent coding system whose complexity - and thus specificity - increases exponentially with a linearly increasing investment (length of address). From one perspective, this chemospecificity represents a storage medium, the very biochemical hard drive that stores our cellular binaries; from another, however, it represents a strong mechanism for assembly programming. It is this set of properties, that of “sticky” strands that bind specifically to their exact complement that will be used along with the basic geometry of helical DNA in various assembly methods.

The structure of DNA alludes to a paradigmatic distinction between modes of binding but, in order to capture the uniqueness of the variety of modes within which DNA-based assembly can occur, it is important to discuss what motivates the categorizing of bonds and then what distinguishes supramolecular behaviour. Labeling bonding as ionic, covalent, or dispersive rises naturally from the observation of vastly differing properties but proves insufficient in more complex systems. The truth, as always, lies somewhere in the middle: electrons exist statistically in a compromise of states. Broad labels allow for the intelligent discussion of molecules without constant reference to the probability  $|\text{kets}\rangle$  of quantum mechanics, but there are forces and systems, however, that these descriptors are clearly incapable of describing. Supramolecular interactions are thus broadly defined as non-covalent but the distinction is more useful if termed as the vast world of interactive phenomena of intermolecular, macromolecular, guest@host, and other systems governed by such alternative forces as spin-interactions, gravity, mechanics, electromagnetism, capillary forces, hydrodynamics, and entropy all the way to forces as esoteric as those produced by Casimir-type interactions. Supramolecular study informs the chemist as to the diverse set of forces that are always active but have scale-dependent and contextual relevance. By considering them all, systems can be engineered not only more intelligently but in novel ways, and this is shown elegantly in the assembly behaviour of DNA.

Ironically, the mode that should be the most intuitive is considered novel within the context of traditional molecular chemistry. The same mechanics that define our “everyday physics” have been shown to underlie self-templating behaviour in DNA. The

ramifications of double-helical geometry on extrastructural interactions have long been studied but, in using crystal-packing models to estimate biological fitting mechanisms, it has been shown that DNA's geometry enforces fundamental, mechanically restricted regimes on itself in close-packing. Whereas DNA is typically understood as it usually observed *ex vivo* in solution – with the free volume and energy to be flexible and dynamic, biological conditions can imply a much greater rigidity. In order to better understand the nature of *in vivo* self-ordering, crystallographic studies were performed. The result, as shown in *fig 3*, was that packing was largely defined by mechanical groove-backbone interactions. Therefore, at relevant densities, these formations could act as the dominant intermolecular interaction, defining the system within which secondary interactions should be understood.

It is thus that supramolecular considerations become critical in the most primal of studies: intracellular mechanics. By considering interactions beyond those which define the molecule, a novel framework is discovered for understanding DNA dynamics within the cell, where the density can be in the relevant scale<sup>i</sup>. What is important to highlight is the simultaneity and relativity involved in reevaluating the supramolecular system.

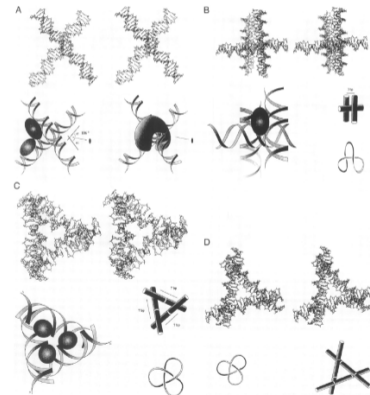


Figure 3 – Modes of geometric packing [i]

Granted an understanding of the importance of geometric fitting, interactions, governed by whatever other forces are relevant, can be modeled more intelligently under this new architectural motif. Enzymatic study, for example, can better understand biochemical interactions given geometric implications on the accessibility of various moieties. By looking at an effect as mundane as mechanical templating, it becomes clear how much the consideration of alternative forces can inform systemic study.

Whereas self-templating can passively define form, Ned Seeman's group at NYU has pioneered an architecture for DNA-based assembly that actively employs GATC base-pair selectivity toward arbitrary, addressable self-assembly<sup>ii</sup>. At its core, the technique relies on nothing but the very same

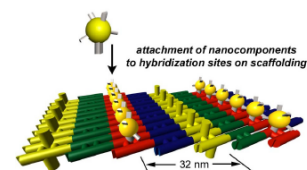


Figure 4 - DNA-capped nanoparticle binding on DNA surface [ii]

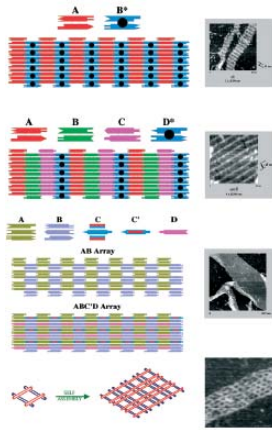


Figure 5 - Construction of DNA lattice [ii]

complementarity that holds every strand of DNA to its partner but, in this case, rather than binding two equal structures, the relationship is radically redefined. DNA crystals are constructed in the “double-crossover” formation where typical double-stranded DNA is made to fold over itself and designed with one strand extending, left to bind to other component blocks either vertically or within the plane. This is the first generation of sticky-assembly that defines a crystal born of sheets of 2D DNA arrays with an arbitrary number of unique DNA blocks. A second generation of sticky-assembly defines

the placement of nanoparticles with DNA-ligands on the scaffolding. By again leaving a single strand to dangle unpaired, this time at the surface, a site for directing the self-assembly of nanocomponents is created with specificity defined by the exposed GATC code. The introduction of nanoparticles synthesized specifically to hold single strands of DNA that are complimentary to those exposed on the surface creates a system that will innately direct the particles to their intended positions on the lattice. Harnessing the natural genetic binding structure on two sequential levels, a convenient system for nanoscale patterning is developed.

The innately nanoscale nature of DNA-based assembly sites is only the beginning to what makes this design for self-assembly so attractive. What distinguishes this technique from most others in nanoscale patterning is the conveniently preprogrammed chemistry that offers highly-selective binding where the level of selectivity and the number of differently selective sites are experimentally designed by the length and uniqueness of the GATC codes employed. Success

in experimental control is elegantly demonstrated in number of experiments where graphics and text are reproduced through DNA-direction<sup>iii</sup>. These images are reminiscent of the classic demonstrations produced by Eigler and Schweizer via the STM-based manipulation of Xenon atoms on a Pt substrate to spell IBM<sup>iv</sup> except that whereas that assembly may have taken 40 GSH (graduate student hours) to arrange, the smiley-face in *fig 6* was accomplished in a single

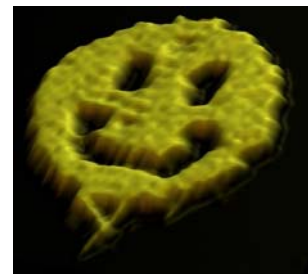


Figure 6 - Smiley-face by DNA assembly [iii]

one-pot reaction.  $10^{13}$  times. Even the results of *Mirkin et al.*, using a massively parallel Dipped Pen Nanolithography (DPN) assembly to create tens of thousands of replicas of Lincoln's topographical portrait<sup>v</sup>, pale in comparison given the tremendous mechanical difficulties with DPN systems and the various practical limitations on using various types of "inks", resolution, etc. Second-order DNA-based self-assembly easily surpasses these limitations and offers a parameterized addressability.

The nature of these critical design considerations have been carefully considered by *Park et al.* in expressing two opposing architectural paradigms: minimal sequence set (MSS) and minimizing depth (MD) in assembly<sup>vi</sup>. The profundity of their study is not in any tremendous progress in terms of the assembly product but rather in the way they reconsider the design of the self-assembly system as a problem in engineering that can be optimized by considering

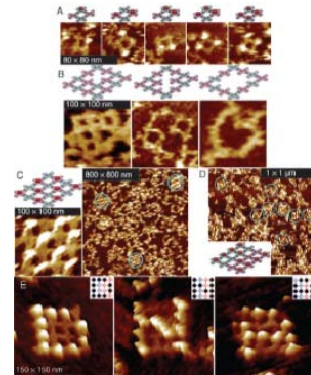


Figure 7 - DNA-based assembly of 'D' 'N' 'A' [vi]

the trade-off between address-space and generations of assembly. As discussed before, the number of available addresses rises exponentially with the effective length of the GATC code involved in binding (the concept of length is tempered by the possibility of partial binding); chemoselectivity implicitly defines the number of sequential assembly steps required for a given pattern, thus defining an inverse correlation between synthetic complexity/selectivity and complexity in assembly. By compromising between the two strategies, the group proposes an efficient system that allows for some degree of reuse while maintaining a high quality of assembly.

Such explorations in patterning have primarily focused on planar structures due to their relative simplicity and their focal role in the micro/nanoelectronics sectors but, as industry has shifted its focus, so too have experimentalists. In fact, the same basic concept of sticky-links can be extended not just to higher orders of sequential assembly but higher dimensional orders of assembly<sup>vii</sup>. By

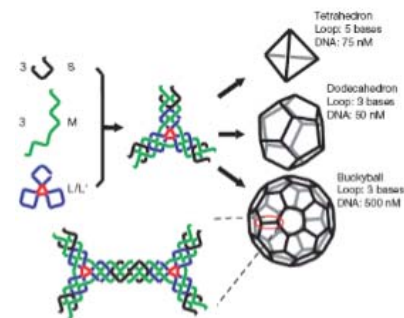


Figure 8 – Steps in self-assembly of 3D DNA structures [vii]

introducing a clever combination of three different types of DNA, the single-strands self-

assemble into a flexible, planar three-point star motif that, in turn, self-assemble into different 3D forms depending on the concentration of DNA in solution. The ultimate polyhedra manifest the exact same GATC-complementarity-based selective binding that was

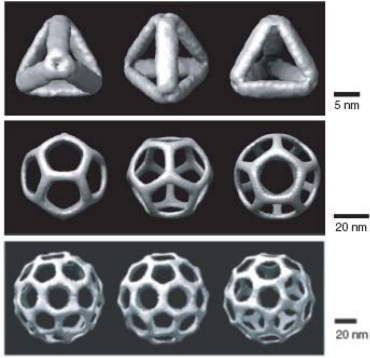


Figure 9 - Reconstructed images of 3D DNA structures [vii]

exhibited in the 2D case, except now there is a second important supramolecular consideration in addition to the ensemble hydrogen-bonding: the structural dynamics of the planar DNA assemblies. *He et al.* show that, in addition to appropriately strong and precise sticky-end-to-end bonding, assembly in 3D implies criteria as to the mechanical properties of the components, namely in terms of strength in bending and resilience. The points of each star have to be able to

bend sufficiently to connect to the next vertex and maintain their integrity under the constant residual stress that this entails; as the number of faces polyhedral faces increases, the required flexibility decreases but the statistical volume for failure increases. Higher-order order supramolecular behaviour is thus partially dependent on the covalent bonding that defines the polynucleotide backbone! Once again, a broader regard of the forces that govern DNA assembly has elucidated both new aspects of known behaviour and opened entirely new avenues for design.

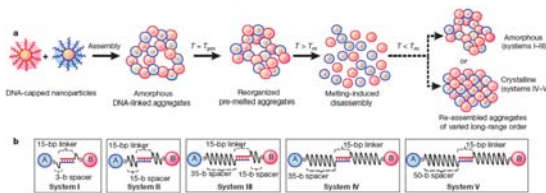


Figure 10 - Procedure in propelling the self-assembly of DNA-capped nanoparticles into crystalline structures [viii]

Perhaps the most exciting illustration of DNA-based supramolecular assembly comes in combining the concepts of sticky-assembly chemistry and dynamic constituents with fundamental thermodynamics. *Nykypanchuk et al.*, have

shown that the careful design of a pair of complementarily DNA-capped nanoparticles can result in a mixed system that can be coaxed to assemble into a B.C.C. crystalline structure<sup>viii</sup>. In perfect parallel with traditional materials

processes, the material is melted down to a random state and

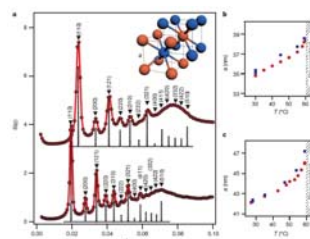


Figure 11 -Structure factor values for assembled crystals [viii]

then slowly quenched to allow for ordered assembly of its species; replacing phase change with thermal denaturation - whereby ensemble hydrogen-bonding is dominated by random thermal energy – and critical size solid nucleation with the reintroduction of transient sticky-bonding, alloy crystallization has been brilliantly recreated using DNA-functionalized nanoparticle constituents. What’s most amazing is the nature of these bonds: even once

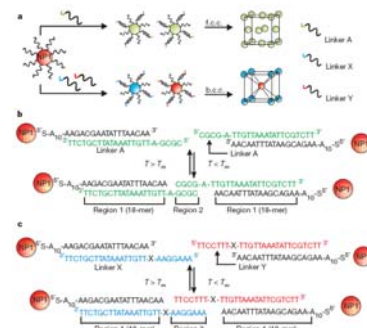


Figure 12 - Assembly mechanism for Mirkin-style DNA-bound crystals [ix]

“crystallized” the GATC bonds are still tenuous, forming and reforming at a tremendous frequency. They stabilize because of the ensemble effect of all those hydrogen-bonds acting between their eight nearest neighbours. Even more control is demonstrated by Mirkin’s group, where they are able to select the Bravais lattice by varying the types of linkers<sup>ix</sup>. Depending on the number of different constituent species, they are able to assemble single-species F.C.C. crystals and two-species Cesium Chloride crystals. By operating within the relevant energetic regime, assembly can be provoked and customized to an astonishing degree.

It this notion of understanding systemic interactions in terms of the superposition of disparate forces of relative importance that supramolecular chemistry is ultimately most powerful in informing. Throughout the study of various modes of DNA-based assembly, paradigms of mechanical templating, sequential selective base-pair bonding in constituent assembly, higher dimensional self-assembly, and mixed-constituent biased thermodynamic assembly have all been demonstrated, together relying on a wide range of supramolecular interactions. It is clear that as cognizance of relevant forces is enhanced so too is the ability to understand and control complex chemical systems.

<sup>i</sup> Timsit *et al.* EMBO Journal, 13, 12, 2737-46 (1994). [13]

<sup>ii</sup> Seeman *et al.* Rep. Prog. Phys, 68, 237-2708 (2005). [21]

<sup>iii</sup> Rothemund, <http://www.dna.caltech.edu/~pwkr/>

<sup>iv</sup> D.M. Eigler and E.K. Schweizer, Nature 344, 524 (1990).

<sup>v</sup> Mirkin *et al.* Nanotechnology, 13, 212-7 (2002).

<sup>vi</sup> Park *et al.* Angewandte Chemie, 45, 735-9 (2006). [5]

<sup>vii</sup> He *et al.* Nature, 452, 198-201 (2008). [12]

<sup>viii</sup> Nykypanchuk *et al.* Nature, 451, 549-52 (2008). [17]

<sup>ix</sup> Park *et al.* Nature, 451, 553-6, (2008). [18]