Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Designing ordered nucleic acid self-assembly processes

Rebecca Schulman[1] and David Doty[2]

A major goal of self-assembly research is the synthesis of biomolecular structures with diverse, intricate features across multiple length scales. Designing self-assembly processes becomes more difficult as the number of species or target structure size increases. Just as the ordered assembly of a machine or device makes complex manufacturing possible, ordered (or 'algorithmic') biomolecular self-assembly processes could enable the self-assembly of more complex structures. We discuss the design of ordered assembly processes with particular attention to DNA and RNA. The assembly of complexes can be ordered using selective, multivalent interactions or active components that change shape after assembly. The self-assembly of spatial gradients driven by reaction-diffusion can also be ordered. We conclude by considering topics for future research.

Addresses
[1] Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, United States
[2] California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, United States

Corresponding author: Schulman, Rebecca (rschulm3@jhu.edu)

Current Opinion in Structural Biology 2015, **31**:57–63

This review comes from a themed issue on **Theory and simulation**

Edited by **Claire Lesieur** and **Klaus Schulten**

http://dx.doi.org/10.1016/j.sbi.2015.03.003

Introduction

While significant progress has been made toward the self-assembly of complex, synthetic biomolecular structures [1–3], the complexity and functionality of these structures are still dwarfed by the complexity and functionality of the structures assembled by organisms. For example, animals can have billions or more ordered features across 12 orders of magnitude in size: Fundamental advances are needed to design and synthesize structures whose complexity compares to those of living things. One potential route to self-assembling structures of significantly greater complexity than is possible currently is to design self-assembly processes by modeling them after the way macroscale machinery is designed and built. Typically, cars or computers are assembled using well-defined, sequential processes. Considered generally, this notion of a set of well-defined sequential steps can be applied not only to building, but also to process design or information processing. In each case, such a well-defined recipe is referred to as an *algorithm*. Importantly, this usage of the word 'algorithm' does not refer to software run on an electronic computer to help design self-assembling systems. This algorithm is the set of steps *biomolecules themselves* follow to assemble a given structure. The idea that biomolecules could execute an algorithm to self-assemble complex structures is supported by recent studies of self-assembly processes in biology: one of their hallmarks is the control of assembly kinetics over multiple assembly steps [4••,5,6].

To design an algorithm for the self-assembly of a biomolecular structure, it is necessary to design both the structure of what is to be assembled and the *sequence of self-assembly steps* expected to produce the desired structure. We will refer to a self-assembly process designed in this way as an ordered self-assembly process. In contrast to designing a set of components that stably form a desired structure at equilibrium, designing an ordered assembly process means that the kinetics of assembly must be understood and optimally, explicitly designed. In practice, characterization and determination of assembly kinetics is more difficult than equilibrium analysis and design because the latter requires only characterizing the minimum energy states of the systems, whereas the former requires characterizing all possible states of the system, and the transition rates between them. Experimental characterization of rapid kinetic transitions can also be technically challenging. Yet despite these potential obstacles, it is become increasingly possible to approach these problems and to do so with an eye to scaling the complexity and functionality of the structures being assembled. Further, the scaling and design of ordered assembly processes can be addressed by considering assembly processes as algorithmic and applying powerful tools for algorithm design from computer science.

This article describes progress toward designing ordered self-assembly processes for DNA and RNA components that assemble via Watson–Crick hybridization, with an emphasis on the application of algorithmic ideas from computer science to enable processes to be scaled. By scaling, we refer to scaling both the size of the assembly (so that it extends across multiple length scales) and the complexity with which the components are arranged (which may be measured in a variety of ways). An increase in either of these metrics may also require that the

number of different types of components (species) used to self-assemble a structure also increase.

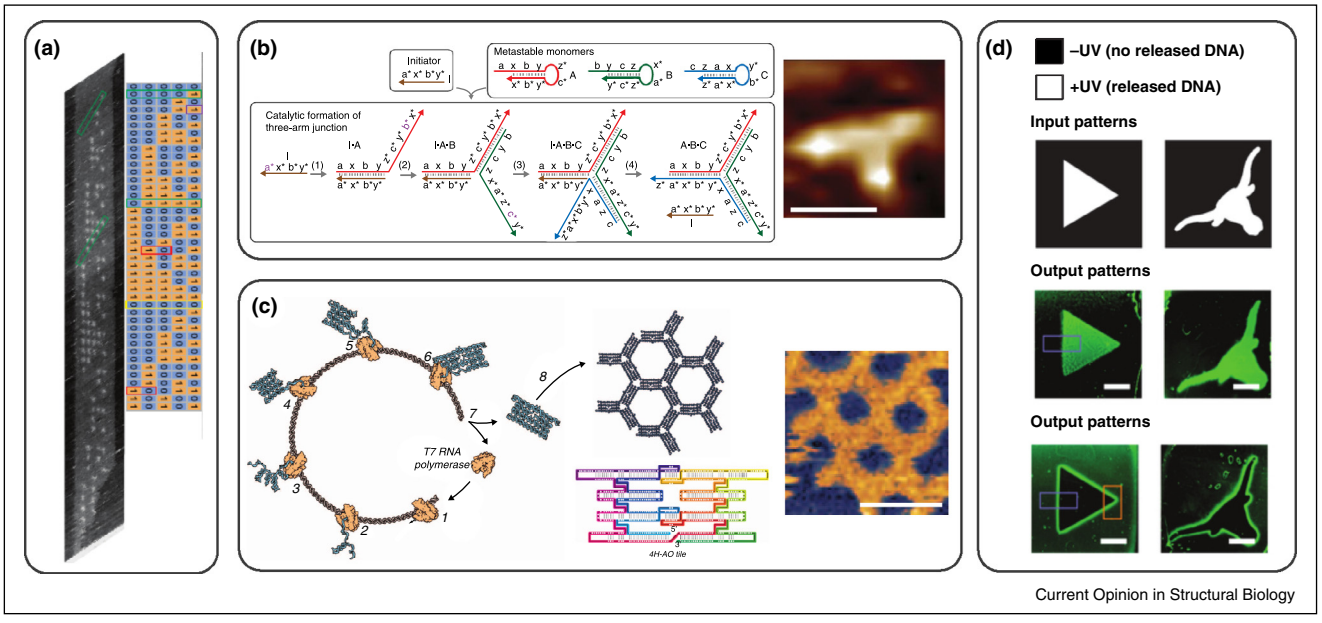
Watson–Crick hybridization processes are particularly amenable to scalable design because base pairing interactions are relatively easy to model and predict computationally [7]. It is also often possible to scale the number of components in a process by using many different components with similar or identical architectures but different sets of complementary subsequences. Because these different subsequence pairs assemble specifically and can have almost identical structures, it is feasible to design large structures in which the order of interactions between the components of the assembled structure are controlled. Examples are shown in Figure 1. In this article we describe two of these examples — algorithmic tile self-assembly and reaction diffusion systems — in detail and touch on related work.

Algorithmic tile self-assembly

Algorithmic tile self-assembly was first described by Winfree [13] as a mechanism for assembling aperiodic crystals from different types of DNA monomers that could cocrystallize [8]. Winfree showed that the assembly of these crystals could be viewed as analogous to the execution of a type of computer program, called a cellular automaton [14]. This view of the assembly process is powerful because it makes it possible to assemble large, complex structures with only a few types of components in a one pot reaction. Algorithmic tile self-assembly processes have been used to assemble fractal structures [15,16] and nanoscale circuit diagrams [17], to design self-replication processes [18] and could in principle be extended to assemble structures of arbitrary complexity and size in one pot reactions.

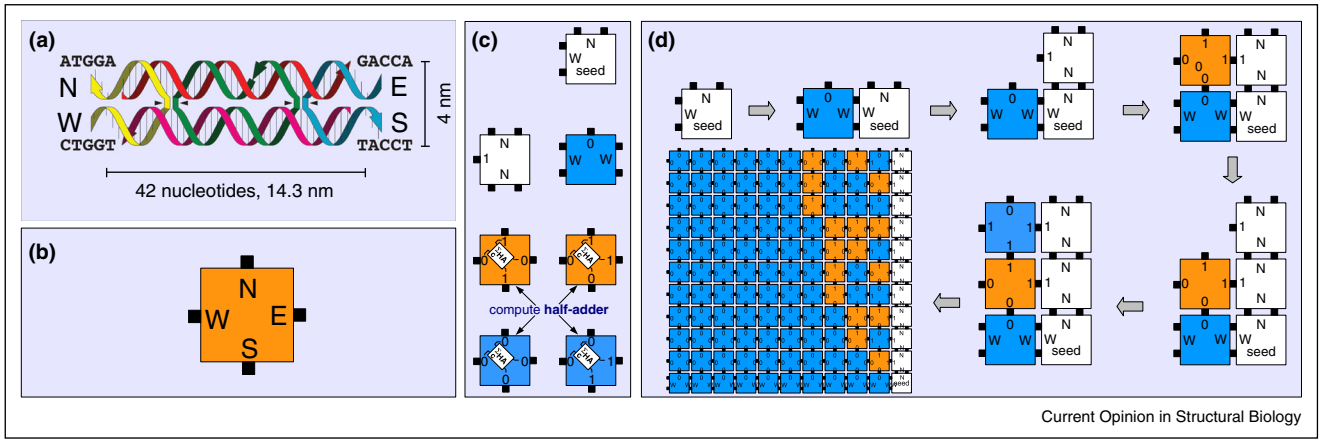
The ordered execution of programmatic steps during assembly is made possible by experimental conditions in which a monomer attaches favorably to two or more binding sites on a crystal but unfavorably to only a single binding site. In algorithmic assembly literature, the term *cooperative binding* is used to describe this effect. The biochemistry literature sometimes uses the term *avidity* to describe this effect, and reserves 'cooperative binding' for a different usage (to describe how binding affinity of a ligand to a substrate increases nonlinearly with ligand concentration).

Figure 1



DNA/RNA structures produced using processes in which the order of assembly is designed and controlled. (Figures taken from respective citations.) **(a)** Algorithmic tile self-assembly of double-crossover DNA tiles [8]. Here, monomers assemble into an aperiodic crystal structure. The example shown is a 'binary counter' in which the dots in each row encode incrementing numbers in binary [9]. (Width of structure ≈100 nm.) The structure is assembled from bottom to top, such that each tile that attaches matches two binding sites in the structure, effectively integrating information about the structure's current assembly state (Figure 2). In other words, assembly steps perform simple computations. **(b)** Catalytically triggered self-assembly of a three-arm junction from DNA hairpin components [10]. (Scale bar: 10 nm.) Although a three-arm junction is the thermodynamically most stable state, a large energetic barrier prevents the formation of the structure until a catalyst strand triggers its assembly. A cascade of trigger and release steps can be used to order an assembly process. **(c)** Co-transcriptional folding of an RNA structure. Secondary structure formation occurs more quickly than transcription so that existing complementary domains will hybridize first and will not interact with domains transcribed later. The order of transcription therefore controls the order of self-assembly. Tile components subsequently assembly into a hexagonal lattice [11••]. (Scale bar: 100 nm.) **(d)** A transformation of a concentration pattern of DNA components, driven by a designed reaction diffusion process [12•]. While multiple reactions and diffusion processes are occurring at once, a separation of time-scales of reaction and diffusion processes produces a well-defined pattern of chemical concentrations. (Scale bar: 3 mm.)

Figure 2



The abstract Tile Assembly Model (aTAM) is a simplified model of an assembly process involving many different types of DNA nanostructures (or 'tiles') that can assemble together into lattices via hybridization reactions involving short single-stranded regions on the tiles, known as sticky ends (or in the aTAM, glues). The goal of the aTAM is to make it possible to design a set of tiles that, assuming that crystallization kinetics generally follow a set of rules that determine the order of reactions, correctly assemble into a designed shape. **(a)** A typical DNA tile used in an algorithmic tile self-assembly process. Each colored ribbon represents a strand of DNA. The sequences of single-stranded regions, or sticky ends, determine which tile types can interact. Complementary sticky ends can participate in binding reactions, whereas noncomplementary sticky ends do not interact. **(b)** An abstract representation of a tile like the one in (a), as it is represented in the aTAM. A tile is modeled as a square with a *glue* on each side that denotes the type of sticky end present. Only matching glues can interact. **(c)** An example *tile set* considered by the aTAM. Each glue has an integer *strength*, depicted by the number of black squares on a side. **(d)** An example growth process in the aTAM involving the tiles in (c). An assembly process starts from a seed tile; another tile can bind to a growing assembly if, and only if, it binds with total strength at least 2, which means either a single strength-2 glue, or two cooperating strength-1 glues (the latter condition idealizing cooperative binding). Any ordered assembly process in which each tile addition follows these rules is allowed. Assembly is considered successful when all possible assembly processes produce the desired final structure. For this particular tile set, cooperative interactions between the `0` and `1` labels on south and east glues of the bottom four tiles in the tile set and matching glues on a growing assembly can simulate the execution of a 'half-adder' Boolean function (interpreting the south and east glues of a tile to be inputs to the function and the other two glues on the same tile to be outputs). Many evaluations of this function during assembly lead the growth of the structure to produce a crystal whose components appear to 'count' in binary: the *n*th row represents the number *n* in binary (starting the count at 0).

While a preference for cooperative interactions can be used to order assembly steps with high probability [19,15], one challenge in algorithmic tile self-assembly is that it is difficult to achieve conditions where this preference is strong enough to ensure that assembly occurs with few or no errors [15,17,9].

The abstract Tile Assembly Model

Because both the programming of a tile assembly process and the chemical kinetics of an algorithmic tile self-assembly process can be difficult to understand, it is helpful to separate the problems of first, designing algorithmic tile self-assembly processes as programs, where it is assumed that *only idealized cooperative interactions* can occur (formally, this means that a monomer can bind to a polymer if and only if at least two of its binding sites match) and second, analyzing how reliably self-assembly occurs when stochastic events may violate this constraint (e.g. when a monomer erroneously binds when only one of its binding sites matches).

The *abstract Tile Assembly Model* (aTAM) [19] addresses concern (i), and is described in Figure 2. The aTAM considers the assembly of basic components called *tiles*; the assembly is considered *algorithmic* because certain binding reactions between tiles are automatically allowed or disallowed by the growth process itself, thus carefully controlling the assembly.

An assembly step can be viewed as a computation because when a tile binds cooperatively to growing crystal, it binds via two binding sites (glues); these binding sites can be viewed as *inputs* to a function. Its two other glues — which will serve as binding sites for future attachments — can then be viewed as the *outputs* of the function. In Figure 2d the function computed in this manner is a *half-adder*: the input bits from the south and east glues are summed to create a two-bit number (00, 01, or 10), with the least significant bit of the sum appearing on the north glue, and the most significant bit of the sum (i.e. the carry) appearing on the west glue. Multiple binding events using this function produce a complex pattern in which the rows of the assembly appear to 'count' in binary, if blue tile types represent 0 and orange tile types represent 1.

Algorithmic tile self-assembly processes can make it possible to assemble many complex objects efficiently (in terms of the number of components) by exploiting complex patterns in the final product or by transmitting

information during an assembly process that can be used to guide and pace assembly. The aTAM makes it possible to explore these issues without considering the complexities of crystallization kinetics. For example, while square and cubic objects of fixed size can be assembled by using a different tile for each position [20,21], aTAM studies suggest that by controlling assembly order, it may be possible to assemble such structures using only about $\log_2 n$ unique tile types (each of which appears in several positions through the final assembly) that self-assemble into an $n \times n$ square [22]. Other studies suggest that algorithmic tile self-assembly serves as a general-purpose 'programming language' for assembling shapes: any shape (sufficiently re-scaled) can be assembled by a number of tile types close to the number of bits required to describe the shape by a computer program [23].

Comparison of the *computational power* of different assembly models can serve as a guide for the design of future assembly processes. For example, two basic assumptions of algorithmic tile self-assembly in the aTAM are that an assembly always nucleates from a seed tile, and that single tiles then attach to a growing assembly. But molecular processes could be designed to allow for different types of interactions. 'Hierarchical assembly,' in which any two tiles or assemblies that interact strongly enough to attach, can be more computationally sophisticated than the original aTAM [24••]. However, hierarchical assembly is in another sense weaker, in that its dynamics cannot emulate those of other hierarchical self-assembly systems [25]. In contrast, there is a single tile set in the original aTAM that can emulate the dynamics of any other system in the original aTAM [26], but it relies crucially on cooperative binding [27•].

The kinetic Tile Assembly Model

While the aTAM predicts that complex patterns can be produced efficiently using algorithmic tile self-assembly, these patterns can only be assembled in practice if cooperative assembly can correctly order the assembly process in line with the aTAM's assumptions. Designed to investigate this question, the kinetic Tile Assembly Model (kTAM) models the assembly process using stochastic chemical kinetics. In the kTAM, tiles attach to assemblies at a rate proportional to the concentration of a particular tile type and independent of temperature, and tiles detach from assemblies at a rate exponential in $-\Delta G^\circ/RT$, where ΔG° is proportional to the total glue strength between interacting tiles, R is the universal gas constant, and T is absolute temperature [19]. Under these assumptions, there are regimes close to assembly melting temperature in which assembly is predicted to occur almost exactly as the aTAM predicts, that is, with few errors (defined as tiles that attach without forming bonds of strength at least 2), but very slowly [19]; reducing the temperature for a given the concentration of a particular tile type speeds up assembly but increases the error rate.

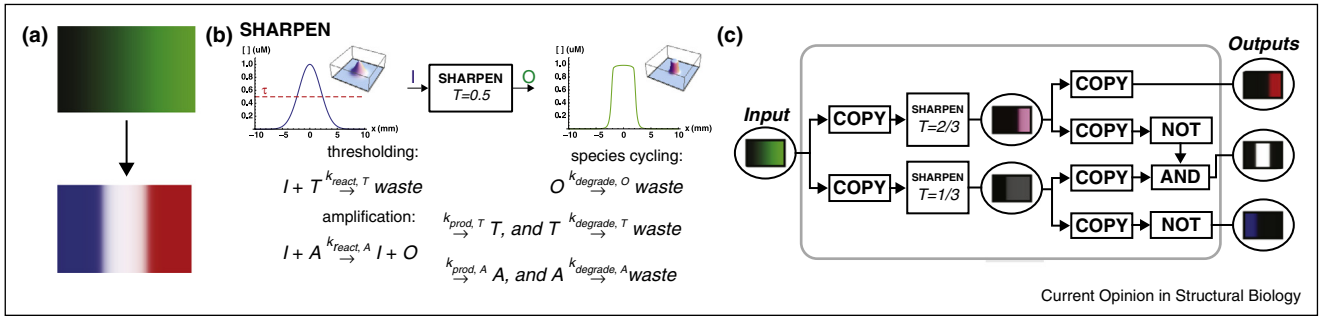
The kTAM also makes it possible to develop methods for designing tiles for which errors are intrinsically less likely to occur under any set of physical conditions for assembly. Proofreading [28–31] has been proposed as a strategy which allows one to transform a given set of tiles into a more complex set of tiles that make fewer errors during assembly. In a proofreading transformation, each component is replaced by a block of new tile types. In experiments, proofreading tile sets based on these models have reduced errors during both assembly growth [32,17,18] and nucleation [33].

Active self-assembly

In algorithmic tile self-assembly a complex assembly process is ordered because only cooperative (or multiple simultaneous) attachments between components and a growing structure are allowed. The opportunity to make grow via multiple attachments arises only when assembly grow reaches particular states. An alternative approach to ordering assembly is *active assembly*, in which components change conformation upon binding, which in turn alters other components in the assembly react with them. While this type of mechanism is common *in vivo*, a systematic consideration of this idea for designing DNA-based assembly processes and the design of synthetic structures with this capacity has begun only recently. The best explored mechanism for active assembly using DNA components are mechanisms that employ strand displacement reactions to trigger downstream self-assembly reactions by activating binding sites on previously inactive tiles [34]. Branched DNA strand structures [10] or a tetrahedron [35] may be assembled from single DNA strands which unfold as the reaction proceeds. The unfolding process controls which assembly steps can occur when. Possible mechanisms for implementing more generalized active components include strand displacement reactions on DNA tiles [34] or helper enzymes [36].

While the computational implications of active assembly are just beginning to explored, recent results indicate that at least in principle, active self-assembly may improve both the efficiency and accuracy of complex assembly processes as compared to passive self-assembly mechanisms. Certain shapes can be assembled with fewer component types [37], and some shapes that cannot be assembled at all in the aTAM can be assembled by active tiles [37,38]. General-purpose computation can also be done with fewer *total* components [37], since several steps of computation can use tiles that are recycled by being broken off from an earlier assembly by deactivating some bonds. The use of active tiles also means that general-purpose computation can be done without *requiring* the use of cooperative binding as in most aTAM results [37,39]. Active self-assembly may also make it possible to design dynamic self-assembly processes, such as self-replication: starting from a 'template' assembly containing a pattern, the pattern can be replicated by growing a

Figure 3



Example programmed reaction-diffusion pattern transformation (Images courtesy [44•]). Here a structure is a stable set of concentration gradients of different species. **(a)** Initial input pattern and final pattern. Colors and color brightness illustrate the concentration of different species. Initially, all species except the input species are homogeneously distributed across the substrate. **(b)** A pattern transformation process consists of a series of reactions divided into modules, with an example module shown here. The output of a module is a transformation of the pattern of the input species. **(c)** A pattern transformation 'circuit' consisting of many modules to perform the transformation shown in (a). The input is not affected by the process of producing the output pattern.

copy on the original and then using signals to deactivate bonds to break the two copies apart [40].

Reaction-diffusion patterning

The DNA lattices produced by an algorithmic tile self-assembly process are crystals whose structure is determined by the precise arrangement of the component monomers (Figure 1a–c). Algorithmic design can also be used to design processes to produce other kinds of ordered arrangement, such as spatial concentration gradients of soluble molecules (Figure 1d.) Gradients as a form of spatial organization are common in living systems as, for example, concentration gradients of morphogens or growth factors [41].

Coupled reaction-diffusion processes have been studied computationally as pattern formation processes for more than 50 years [42]. While in principle, controlling the rate of each of the reaction and diffusion processes in these systems can enable controlled pattern formation, it has been difficult to build systems based on these principles such that they can be programmed and scaled [43].

Recently, advances in the *de novo* design of chemical reaction networks based on interactions between synthetic DNA complexes [45,46,36] has provided a new method of building complex systems for pattern generation akin to the complex networks seen in multicellular systems. Diffusion rates for DNA molecules are also well-characterized [47], which could be exploited for patterning.

Particularly amenable to engineering and scaling is the process of pattern transformation, the formation of a well-defined pattern given a well-defined reaction-diffusion network and a well-defined initial, generally simpler pattern of concentrations. An initial demonstration of DNA-driven pattern transformation showed the transformation of an initial pattern (produced using photolithography which activated a DNA species in some areas of the environment but not others) into a second pattern with a well-defined 'edge' [12•]. Reactions only happened at boundary regions where multiple reactants could encounter one another. Slightly more elaborate patterns could be produced by building multiple possible reactions between components with different diffusion rates. Another DNA-based reaction-diffusion patterning system for the production of a traveling wave of molecules driven by a set of autocatalytic reactions was also recently demonstrated [48••].

In order to scale the complexity of patterns that can be produced, new design and simulation techniques are needed [49] so that designs for scalable systems, such as modular reaction-diffusion networks, in which new features can be produced by chaining modules together [44•,50] can be implemented and scaled (Figure 3).

Conclusions

The design of kinetic pathways for assembly processes and flexible design frameworks that allow for robustness and scaling are underway in different areas of DNA–RNA-based self-assembly design. An important challenge will be integrating modeling and design with experimental approaches. In the future, an understanding of assembly kinetics could make it possible to address assembly problems in nanostructures that are currently designed using thermodynamic methods, including DNA origami structures [51,52] and single-stranded tile structures [20,21]. Recent work focuses on optimizing assembly conditions in order to produce the desired lowest energy structure [53], but the assembly processes producing these structures are still poorly understood.

Future algorithmic tile self-assembly research must address the challenges inherent in reaction ordering using

cooperative binding. While many models of complex self-assembly processes have been proposed, high error rates in experiments mean that scaling assembly processes to implement most of these ideas will be difficult or impossible without new process improvements. Perhaps a more viable alternative for the design of self-assembly processes is to explore alternative assembly models for a variety of architectures and at a variety of scales. For example, active assembly processes may make nanoscale assembly more robust, and reaction-diffusion processes may be appropriate for inducing spatial organization at the millimeter scale.

In addition, the explicit design of assembly processes for nucleic acid components may be increasingly important for assembly processes in complex environments, where it may not be easy to reach equilibrium and many rates of interaction may be unknown, such as *in vivo* or in confined environments. Self-assembly process design and an understanding of how a particular environment changes the rates and mechanism of component interactions could together produce a systematic method of constructing novel structures in such complex environments.

As of today, the majority of research into the design of ordered self-assembly processes has been focused on DNA and RNA self-assembly processes, because hybridization interactions between these molecules are highly specific and the rate constants between components can be chosen to some extent by design. However, as more information about rate constants for protein interactions becomes available and *in silico* models of protein improve, we expect that these ideas could be extended to apply to protein self-assembly as well, and in principle to other molecules for which both kinetic and thermodynamic parameters are available. The ability to apply these ideas to a diverse set of biomolecules will be an important step toward the development of principles self-assembly techniques that make it possible to synthetically recapitulate the complexity of biological complexes and molecular machines.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

R.S. acknowledges grants NSF-CCF-1161941, a Turing Centenary Fellowship for support and DE-SC010595 for salary support in writing this article. D.D. acknowledges NSF grants CCF-1219274 and CCF-1162589 and the Molecular Programming Project under NSF Grant 1317694.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Pinheiro AV, Han D, Shih WM, Yan H: **Challenges and opportunities for structural DNA nanotechnology**. *Nat Nanotechnol* 2011, **6**:763-772.

2. King NP, Sheffler W, Sawaya MR, Vollmar BS, Sumida JP, André I, Gonen T, Yeates TO, Baker D: **Computational design of self-assembling protein nanomaterials with atomic level accuracy**. *Science* 2012, **336**:1171-1174.

3. Gröschel AH, Walther A, Löbling TI, Schacher FH, Schmalz H, Müller AHE: **Guided hierarchical co-assembly of soft patchy nanoparticles**. *Nature* 2013, **503**:247-251.

4. •• Marsh JA, Hernández H, Hall Z, Ahnert SE, Perica T, Robinson CV, Teichmann SA: **Protein complexes are under evolutionary selection to assemble via ordered pathways**. *Cell* 2013, **153**:461-470.
This article suggests that well-defined, ordered assembly pathways are of importance in biological self-assembly, perhaps because they make assembly more efficient.

5. Fletcher DA, Mullins RD: **Cell mechanics and the cytoskeleton**. *Nature* 2010, **463**:485-492.

6. Davidson EH: **Emerging properties of animal gene regulatory networks**. *Nature* 2010, **468**:911-920.

7. Zadeh JN, Steenberg CD, Bois JS, Wolfe BR, Pierce MB, Khan AR, Dirks RM, Pierce NA: **NUPACK: analysis and design of nucleic acid systems**. *J Comput Chem* 2011, **32**:170-173.

8. Winfree E, Liu F, Wenzler LA, Seeman NC: **Design and self-assembly of two-dimensional DNA crystals**. *Nature* 1998, **394**:539-544.

9. Evans C: *Crystals that Count! Physical Principles and Experimental Investigations of DNA Tile Self-Assembly (Ph.D. thesis)*. Pasadenia, CA: California Institute of Technology; 2014.

10. Yin P, Choi HMT, Calvert CR, Pierce NA: **Programming biomolecular self-assembly pathways**. *Nature* 2008, **451**:318-322.

11. •• Geary C, Rothemund PW, Andersen ES: **A single-stranded architecture for cotranscriptional folding of RNA nanostructures**. *Science* 2014, **345**:799-804.
The components of this self-assembly reaction are produced by transcription, making it possible to control when components are available for assembly.

12. • Chirieleison SM, Allen PB, Simpson ZB, Ellington AD, Chen X: **Pattern transformation with DNA circuits**. *Nat Chem* 2013, **5**:1000-1005.
A demonstration that DNA-based chemical reaction networks could be used for patterning reaction-diffusion systems.

13. Winfree E: **On the computational power of DNA annealing and ligation**. In *DNA Based Computers.* Edited by Lipton RJ, Baum EB.. **DIMACS**. Providence, RI: American Mathematical Society; 1996:199-221.

14. Doty D: **Theory of algorithmic self-assembly**. *Commun ACM* 2012, **55**:78-88.

15. Rothemund PWK, Papadakis N, Winfree E: **Algorithmic self-assembly of DNA Sierpinski triangles**. *PLoS Biol* 2004, **2**:424-436.

16. Fujibayashi K, Hariadi R, Park SH, Winfree E, Murata S: **Toward reliable algorithmic self-assembly of DNA tiles: a fixed-width cellular automaton pattern**. *Nano Lett* 2008, **8**:3554-3560.

17. Barish RD, Schulman R, Rothemund PWK, Winfree E: **An information-bearing seed for nucleating algorithmic self-assembly**. *Proc Natl Acad Sci U S A* 2009, **106**:6054-6059.

18. Schulman R, Yurke B, Winfree E: **Robust self-replication of combinatorial information by crystal growth and scission**. *Proc Natl Acad Sci U S A* 2012, **109**:6405-6410.

19. Winfree E: *Algorithmic Self-Assembly of DNA (Ph.D. thesis)*. California Institute of Technology; 1998.

20. Wei B, Dai M, Yin P: **Complex shapes self-assembled from single-stranded DNA tiles**. *Nature* 2012, **485**:623-626.

21. Ke Y, Ong LL, Shih WM, Yin P: **Three-dimensional structures self-assembled from DNA bricks**. *Science* 2012, **338**:1177-1183.

22. Rothemund PWK, Winfree E: **The program-size complexity of self-assembled squares (extended abstract)**. In *STOC 2000: Proceedings of the Thirty-Second Annual ACM Symposium on Theory of Computing*. 2000:459-468.

23. Soloveichik D, Winfree E: **Complexity of self-assembled shapes**. *SIAM J Comput* 2007, **36**:1544-1569 (Preliminary version appeared in DNA 2004).

24. •• Cannon S, Demaine ED, Demaine ML, Eisenstat S, Patitz MJ, Schweller RT, Summers SM, Winslow A: **Two hands are better than one (up to constant factors)**. In *STACS 2013: Proceedings of the Thirtieth International Symposium on Theoretical Aspects of Computer Science*. 2013:172-184.

This shows how to simulate 'seeded' tile assembly in an environment that allows unseeded growth, using cooperative binding to control how polymers are allowed to attach.

25. Demaine ED, Patitz MJ, Rogers T, Schweller RT, Summers SM, Woods D: **The two-handed tile assembly model is not intrinsically universal**. In *ICALP 2013: Proceedings of the 40th International Colloquium on Automata, Languages and Programming*. 2013.

26. Doty D, Lutz JH, Patitz MJ, Schweller RT, Summers SM, Woods D: **The tile assembly model is intrinsically universal**. In *FOCS 2012: Proceedings of the 53rd Annual IEEE Symposium on Foundations of Computer Science*. IEEE; 2012:302-310.

27. • Étienne Meunier P, Patitz MJ, Summers SM, Theyssier G, Winslow A, Woods D: **Intrinsic universality in tile self-assembly requires cooperation**. In *SODA 2014: Proceedings of the 25th Annual ACM-SIAM Symposium on Discrete Algorithms*. 2014:752-771.

This shows that cooperative binding is necessary to achieve a certain strong form of simulation power in algorithmic tile self-assembly.

28. Winfree E, Bekbolatov R: **Proofreading tile sets: error correction for algorithmic self-assembly**. In *DNA.* Edited by Chen J, Reif JH.. **Lecture Notes in Computer Science**. Springer; 2003:126-144.

29. Chen H-L, Goel A: **Error free self-assembly with error prone tiles**. In *DNA 2004: Proceedings of the 10th International Meeting on DNA Based Computers*. 2004.

30. Chen H-L, Goel A, Luhrs C: **Dimension augmentation and combinatorial criteria for efficient error-resistant DNA self-assembly**. In In *SODA 2008: Proceedings of the Nineteenth Annual ACM-SIAM Symposium on Discrete Algorithms.* Edited by Teng S-H. *SODA 2008: Proceedings of the Nineteenth Annual ACM-SIAM Symposium on Discrete AlgorithmsSan Francisco, CA, USA, January 20–22, 2008: SIAM; 2008:*409-418.

31. Schulman R, Winfree E: **Programmable control of nucleation for algorithmic self-assembly**. *SIAM J Comput* 2009, **39**:1581-1616 (Preliminary version appeared in DNA 2004).

32. Chen H-L, Schulman R, Goel A, Winfree E: **Reducing facet nucleation during algorithmic self-assembly**. *Nano Lett* 2007, **7**:2912-2919.

33. Schulman R, Winfree E: **Synthesis of crystals with a programmable kinetic barrier to nucleation**. *Proc Natl Acad Sci U S A* 2007, **104**:15236-15241.

34. Zhang DY, Hariadi RF, Choi HM, Winfree E: **Integrating DNA strand-displacement circuitry with DNA tile self-assembly**. *Nat Commun* 2013, **4**:1965.

35. Sadowski JP, Calvert CR, Zhang DY, Pierce NA, Yin P: **Developmental self-assembly of a DNA tetrahedron**. *ACS Nano* 2014, **8**:3251-3259.

36. Padirac A, Fujii T, Rondelez Y: **Nucleic acids for the rational design of reaction circuits**. *Curr Opin Biotechnol* 2013, **24**:575-580.

37. Padilla JE, Patitz MJ, Pena R, Schweller RT, Seeman NC, Sheline R, Summers SM, Zhong X: **Asynchronous signal passing for tile self-assembly: fuel efficient computation and efficient assembly of shapes**. In *UCNC 2013: Proceedings of Unconventional Computation and Natural Computation 2013*. **LNCS**. 2013:174-185.

38. Jonoska N, Karpenko D: **Active tile self-assembly. Part 2: Self-similar structures and structural recursion**. *Int J Found Comput Sci* 2014, **25**:165-194.

39. Jonoska N, Karpenko D: **Active tile self-assembly. Part 1: Universality at temperature 1**. *Int J Found Comput Sci* 2014, **25**:141-164.

40. Keenan A, Schweller RT, Zhong X: **Exponential replication of patterns in the signal tile assembly model**. *DNA Computing and Molecular Programming – 19th International Conference, DNA 19; Tempe, AZ, USA, September 22–27, 2013. Proceedings: 2013:118-132*.

41. Ashe HL, Briscoe J: **The interpretation of morphogen gradients**. *Development* 2006, **133**:385-394.

42. Turing AM: **The chemical basis of morphogenesis**. *Philos Trans Roy Soc B* 1952, **237**:37-72.

43. Epstein IR, Vanag VK, Balazs AC, Kuksenok O, Dayal P, Bhattacharya A: **Chemical oscillators in structured media**. *Acc Chem Res* 2012, **45**:2160-2168.

44. • Scalise D, Schulman R: **Designing modular reaction-diffusion programs for complex pattern formation**. *Technology* 2014, **2**:55-66.

This shows that in principle, that reaction-diffusion processes can be ordered temporally, and that this ordering makes it possible to produce complex gradients with features across multiple size scales.

45. Soloveichik D, Seelig G, Winfree E: **DNA as a universal substrate for chemical kinetics**. *Proc Natl Acad Sci U S A* 2010, **107**:5393-5398.

46. Zhang DY, Seelig G: **Dynamic DNA nanotechnology using strand displacement reactions**. *Nat Chem* 2011, **3**:103-113.

47. Smith DE, Perkins TT, Chu S: **Dynamical scaling of DNA diffusion coefficients**. *Macromolecules* 1996, **29**:1372-1373.

48. •• Zadorin AS, Rondelez Y, Galas J-C, Estevez-Torres A: **Synthesis of programmable reaction-diffusion fronts using DNA catalyzers**. *Phys Rev Lett* 2015, **114**:068301.

This shows that the ability to control the reaction and diffusion rates of DNA-based chemical reaction networks can make it possible to programmatically tune the spatial and temporal features of reaction-diffusion patterns.

49. Dalchau N, Seelig G, Phillips A: **Computational design of reaction-diffusion patterns using DNA-based chemical reaction networks.DNA Computing and Molecular Programming 20**. 2014:84-99.

50. Scalise D, Schulman R: **Emulating cellular automata in chemical reaction-diffusion networks.DNA Computing and Molecular Programming 20**. 2014:67-83.

51. Rothemund PWK: **Folding DNA to create nanoscale shapes and patterns**. *Nature* 2006, **440**:297-302.

52. Douglas SM, Dietz H, Liedl T, Högberg B, Graf F, Shih WM: **Self-assembly of DNA into nanoscale three-dimensional shapes**. *Nature* 2009, **459**:414-418.

53. Sobczak J-PJ, Martin TG, Gerling T, Dietz H: **Rapid folding of DNA into nanoscale shapes at constant temperature**. *Science* 2012, **338**:1458-1461.