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Non-complementary computation

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Molecular computing programmed with complementary nucleic acid strands allows the construction of sophisticated biomolecular circuits. Now, systems with partially complementary strands have been shown to enable more compact and faster molecular circuits, and may illuminate biological processes.

Our bodies are computers. From organs, to cells, to molecules, gigabytes of information are constantly being processed by neural networks, immune cascades and gene regulation networks enabling us to exist, thrive and evolve. Unlike electronics, much of biological computing is done with molecules. A simple reaction A + B → C can be made an equivalent of an AND logic gate that gives output C only when both input molecules A and B are present. Other logic gates such as NOT, OR and NAND can also be constructed with chemical reactions implementing (in theory) any computer algorithm. Now, writing in *Nature Chemistry*, Maxim Nikitin describes a mode of biocomputing based on low-affinity molecular interactions[1].

Typically limited by diffusion rates, biocomputing with small molecules, proteins and nucleic acids is much slower than with electrons. So instead of competing with silicon-based computers, molecules have been used to embed computation into nanostructures for 'smart' sensing and therapy in vivo, where electronics are hard to deploy. For example, a large DNA–protein hybrid molecule can be programmed to expose a cell-death-triggering moiety only when two cancer-associated markers (AND gate inputs) are present on a cell surface, which results in much lower off-target toxicity compared with therapies without onboard computation[2].

Another driving force behind the currently blooming biocomputing field is the realization that synthetic DNA and RNA can serve as universal substrates for molecular programming. Simple pairing rules (A–T and G–C) are used to program structure[3] and behaviour[4]. This 'molecular programming' has infinite potential for scalability — similar to the endless variety of computer programs that can be created from 0s and 1s. In addition, many biological signals such as light, temperature and molecules can be converted into DNA inputs, for example, with the help of photoactive molecules, aptamers and advanced thermodynamics models[5].

An extremely powerful mechanism for molecular computing is strand displacement (Fig. 1, left). In this process, an invading strand hybridizes with a short complementary sequence called a toehold. The resulting complex then undergoes branch migration, causing the original bound strand to be released. Using strand displacement, we can make an AND logic gate, where A and B are inputs and C is the output. Beyond a simple AND gate, we can combine logical operations implemented using strand displacement to build complex circuits, such as a square root circuit[6] and even a 100-bit 9-memory neural network[7]. These impressive demonstrations require increasingly larger numbers of strands and become slower with more inputs. Nikitin's mode for biocomputing has significant advantages in terms of the time and number of strands needed compared with strand displacement.

In strand displacement, the toehold and branch migration domains are designed to pair with their fully complementary partners. Rather than using only one perfect fully complementary partner, Nikitin proposes using multiple partially complementary strands in a process he terms 'strand commutation' (Fig. 1, right). He constructed elementary YES, NOT, AND and OR Boolean logic gates using RNA and DNA oligonucleotides, then observed the computation process with a fluorescence spectrometer by using strands with attached fluorophores and quenchers. Fluorescence is low when the fluorophore and quenchers are nearby in a complex, but fluorescence increases when a new partner displaces a strand bearing a quencher (Fig. 1).

By combining simple logic gates, Nikitin further demonstrated complex circuits such as a 3-bit memory and computing the square root of a 4-bit number — common benchmarks in molecular computing. Compared with previous implementations of a square-root circuit requiring 130 strands with 10 hours for computation (with signal restoration)[6] and 37 oligos with 25 minutes of computation (with enzymes and no signal restauration)[8], strand commutation required only 9 short DNA sequences and less than 5 minutes of computation time (but no signal restoration).

As further evidence of the promise of the approach, Nikitin simulated logic gates with an extremely large number of inputs and algebraic computations. In addition to these impressive achievements in molecular computing, another important contribution of this work is a demonstration of how weak non-complementary interactions could explain mechanisms of gene regulation. Using RNase H-mediated degradation of mRNA (messenger RNA) on its binding with the antisense ssDNA (single-stranded DNA) as an example, Nikitin shows that any ssDNA that is non-complementary to the gene can be made into a specific input in a circuit that controls the gene expression.

By moving away from perfect complementarity, strand commutation enables several advantages over strand displacement. Partially complementary pairings are weaker and more reversible compared with fully complementary pairings; this enables faster molecular information relay via rapid strand exchange (although its mechanism was not studied in this paper), and therefore faster computation. Weak reversible binding also reduces the stability of undesired complexes between participating oligonucleotides. Better scalability in the number of inputs can be achieved because the number of partially complementary strands for a specific sequence is much larger than a single perfect complementary strand. Thus, a multitude of strands with non-matching sequences can simultaneously interact with a single strand thereby passing information from multiple inputs, as demonstrated by simulating an impressive 572-input AND-gate. The much larger sequence space of non-matching strands allows finer tuning of binding energies and constructing logic gates with shorter strands, leading to simpler designs. As can be inferred from Fig. 1, scaling to more inputs can be achieved without increasing the length of strands in a gate.

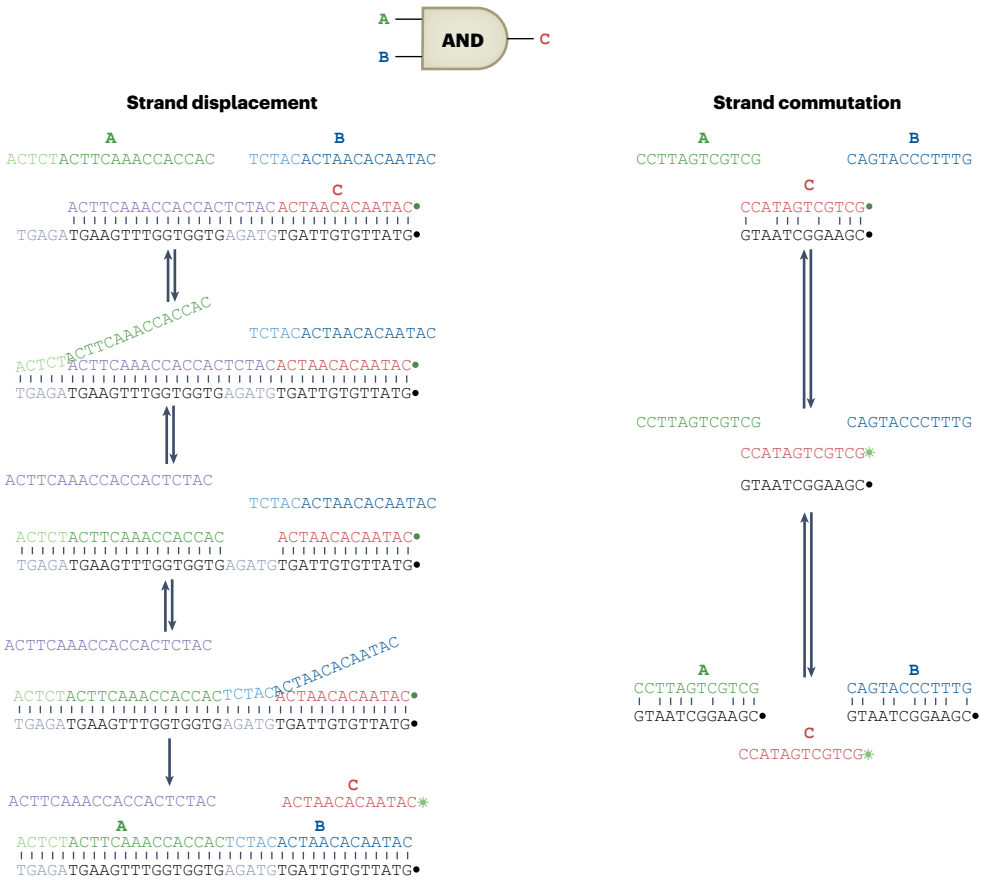

Fig. 1 | An AND logic gate implemented with strand displacement and strand commutation. A and B are inputs and C is the output. The schemes are simplified for clarity. The toeholds are in faded colour. Fluorophore (green dot) is quenched when near the quencher (black dot) and fluorescent (bright green star) when the quencher is removed.

These strengths of strand commutation come with several challenges. In strand displacement, the kinetics and thermodynamics of hybridization can be readily programmed using well-understood sequence design principles. For example, longer toeholds lead to faster reactions[9], while equilibrium dissociation constants can be accurately predicted[5]. Partially complementary interactions cannot be predicted as accurately, so new models will need to be developed. More accurate interaction prediction algorithms will help in designing much larger multistage circuits and storing megabits of information. However, for constructing faster and more complex synthetic circuits, bringing strands closer together on surfaces[10] or in compartments can enable faster computing and many parallel instances with reusable gates requiring a smaller number of unique strands; this is likely to be a more viable alternative to the well-mixed systems studied here.

Apart from a powerful design concept for synthetic molecular computation, the work by Nikitin suggests a framework to better understand natural biochemical processes based on weak interactions. For example, the binding of an antibody — a protein highly tailored for a specific target — to its antigen target is often very strong, off-target binding is heavily selected against, and specificity is high, like traditional molecular computation with longer DNA complexes that use fully complementary sequences carefully designed to minimize interactions with other DNA sequences besides their own. However, Nikitin's mismatch approach allows for a range of interactions with several other species and is more akin to the protein albumin, a multifunctional transport protein binding a wide range of endogenous substances and drugs in the body. As such, it plays a large role in the stability and pharmacokinetic properties of numerous substances affecting the overall homeostasis of our entire body. Whereas nature used evolution to create motifs that interacted at just the right affinities to allow for physiological processes, Nikitin used prediction software. Thus the concepts put forward in this work will help illuminate understudied weak interactions in the genome, proteome and metabolome, and will stimulate new lines of research beyond molecular programming with a double helix.

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Competing interests

The authors declare no competing interests.