Molecular logic and computing

Molecular substrates can be viewed as computational devices that process physical or chemical 'inputs' to generate 'outputs' based on a set of logical operators. By recognizing this conceptual crossover between chemistry and computation, it can be argued that the success of life itself is founded on a much longer-term revolution in information handling when compared with the modern semiconductor computing industry. Many of the simpler logic operations can be identified within chemical reactions and phenomena, as well as being produced in specifically designed systems. Some degree of integration can also be arranged, leading, in some instances, to arithmetic processing. These molecular logic systems can also lend themselves to convenient reconfiguring. Their clearest application area is in the life sciences, where their small size is a distinct advantage over conventional semiconductor counterparts. Molecular logic designs aid chemical (especially intracellular) sensing, small object recognition and intelligent diagnostics.

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Do you remember your first chemistry experiment? Perhaps you poured in a reagent and maybe heated a substance in a test tube. You saw that the initial substance was transformed into the product because it was distinguishable by the change of a property such as colour or physical form (Fig. 1a). This process can be viewed differently, however, through the eyes of a computer scientist (Fig. 1b) whereby the product is an 'output' of a combination of chemical and physical 'inputs'. Naturally, this analogy requires several caveats. For instance, inputs to many simple computational logic devices continually control the output. In a chemical sense, therefore, our choice of reaction needs to be reversible, otherwise each device would only provide one constant output. However, other simple computational logic devices can be latched in a given output state. Some of these have chemical analogues too[1,2].

BOOLEAN LOGIC

Although the semiconductor business brought computation to worldwide attention, computational ideas were available before transistors were fabricated and have a long history[3]. Briefly, the binary number system allows the assignment of 'true' to '1' and 'false' to '0', which is the couching of statements in mathematical terms[4]. The lasting legacy of George Boole's time in Ireland[3] was the method of manipulating these simple numbers with logic operations. Logic gates, which perform such operations, have just one input and one output in their simplest form (Fig. 2a). The NOT gate inverts any binary signal that it receives, as shown in its truth table. The other three (YES, PASS 1 and PASS 0) are considered to be trivial by the semiconductor business, but we will see their utility later in the applications of molecular logic. 2-input logic allows more options, a few of which are shown in Fig. 2b. For example, the AND gate requires both inputs to be '1' before an output of '1' is sent. Importantly, some of these 2-input gates allow the construction of arithmetic processors, which are critical to modern computers. There is a similar history in recognizing computational concepts in biology[5,6], including human behaviour. It is educational to consider the computations required inside your head to recognize someone who approaches you. Many questions are asked about that person's face. Answers are obtained by inspection and compared with stored information, all of this being completed in the subsecond timescale. At the level of biomolecules, proteins of various kinds receive chemical inputs and output product molecules in a process similar to that shown in Fig. 1a. Sometimes, a modification of the protein itself is the output. Such operations occur in their thousands, and often in series, within each of your cells at each moment of your life.

WHY MOLECULES?

Computation with molecules is no less realistic than with well-established semiconductor materials that dominate in today's computers. Although the properties of semiconductors can be tuned by considering size effects or doping strategies, the vast array of chemical reactions that have been developed over the last 150 years allows the synthetic chemist to access a diverse range of molecular structures. In this way, the properties of molecules can be modified rationally in a systematic process, offering a much greater degree of variability and control of their properties. Moreover, assemblies of molecules (supermolecules[7]) containing multiple modules — each with its own function — are particularly promising for information handling because, for example, one module could be used to capture a chemical

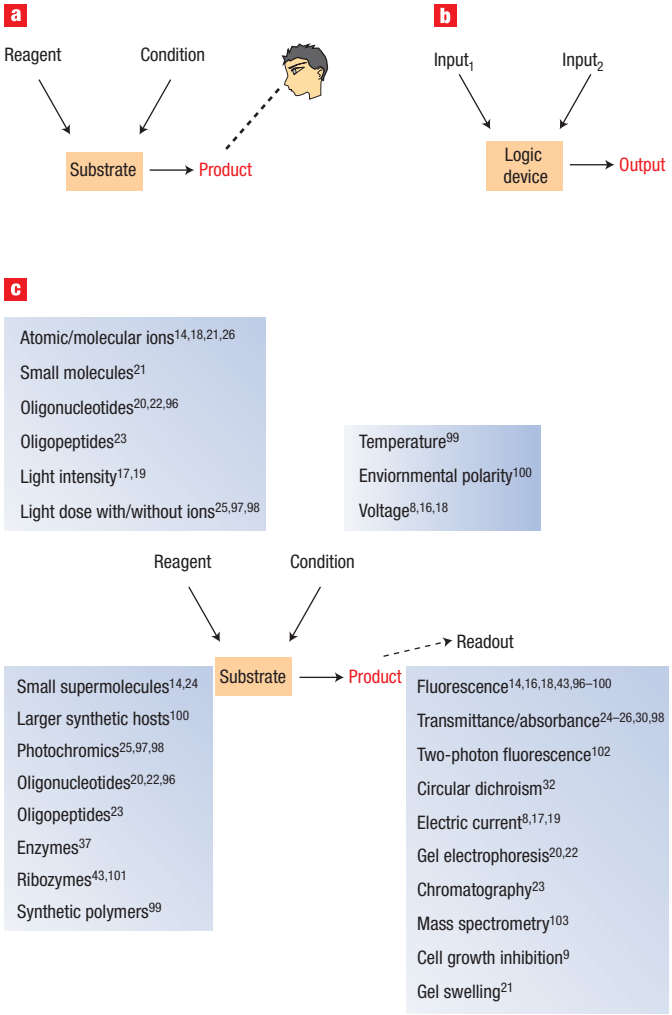


Figure 1 Chemical reactions and computational processes. **a**, A molecular substrate can be treated with a chemical reagent and/or placed under different environmental conditions to generate a product that can be observed because of a change in a physical property. **b**, In a conceptually similar process, a logic device can process a number of inputs to generate a measurable output. Various patterns of electrical inputs entering a computational logic device and producing electrical outputs in various patterns have many similarities (and some key differences) with what goes on in a typical chemical reaction. **c**, A wide range of chemical phenomena and reactions involving molecules, from masses as small as 100 Da and as large as many thousands can now be explicitly interpreted in a logical content. Various biomolecules feature prominently. Photochemical, thermal or electrochemical stimulation is common.

'input' and another would then be triggered to give an output of some kind.

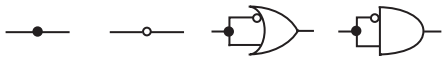
The issue of connectivity is frequently raised when designing nanosized devices and this is the strength of larger semiconductor devices, where the output from one device is used to control another. It is not easy to pass the output from one molecular logic gate to serve as an input to the next because the inputs and outputs differ in nature in many instances. It must be stressed, however, that molecular computation need not follow the semiconductor blueprint. Although one aim may be to make molecular substitutes for semiconductors within existing logic devices[8], this developing field should embrace its differences and follow conceptually different paths. Examples of this will be given at several points during this review.

One example is the integration of biological entities such as cells into synthetic logic systems in which the strengths of semiconductor devices disappear because of issues of size and material incompatibility. Cells process much information concerning internal or external chemicals in order to survive and grow[5,6], which requires intracellular species to be present at the right level in the right place, at the right time. Cell viability will be affected if these conditions are not met by certain species. Logical combinations of 'high' or 'low' levels (compared to the normal) can be arranged, via an integrated synthetic device, to release a therapeutic agent[9] or to generate a light signal for monitoring purposes (see the section on applications). The speed of molecular devices is limited (to millisecond time scales) by rates of diffusion and binding but this is tolerable when biological processes of similar or longer duration are involved. At this level of information processing within living systems, computation — by any means and of any magnitude — becomes significant.

An important consideration when using molecular logic devices is addressing. Semiconductor logic devices have wires along which electrical input signals are sent and other wires along which electrical output signals emerge. Though some molecular counterparts are beginning to be addressed in this way[8], most are addressed differently. For instance, a chemical (atomic or molecular) input signal hits the molecular device by simple diffusion in solution. The output signal emerges as fluorescence light, which can be picked up at a distance. The simplest molecular information processors are sensors and these are already loyal servants of cell biology. For instance, Tsien's sensor, so-called fura-2, measures Ca^{2+} concentration changes[10], which occur in millisecond timescales and in micrometre spaces within living cells during excitation and rest. The private life of intracellular Ca^{2+} is laid bare in a cinematic fashion. Like many other molecular sensors, fura-2 operates at the molecular level and can be addressed optically as a matter of routine. The function of related sensors has even been observed at the level of single molecules[11]. The routine optical addressing happens as follows: a microscope examines the intensity of fluorescence light in a chosen colour (510 nm) from each pixel while exciting the sample with ultraviolet light (335 nm). Furthermore, information about chemical concentrations can be gathered from nanometric spaces near membranes in a similar way by making use of hydrophobic effects to carefully position molecular devices[12]. H^+ concentrations near membranes are responsible for generating ATP (adenosine triphosphate), the energy currency in the cell. Single-device addressing[11], though elegant, is unnecessary for the present purpose if the devices are all in similar environments because they will report the same average information.

Another issue concerns the stability of molecular computational devices. The longevity of inorganic semiconductor materials contrasts with the decay of organic molecules. Yet again, however, the unique opportunities of interfacing molecular computational systems with cellular biosystems mean that such durability is not necessary in order to perform their function. Indeed, many cell imaging experiments carried out over several hours would be served more than adequately by, for instance, fluorescent molecular logic systems. The real-life success of their cousins, fluorescent sensors, was mentioned above. Nevertheless durability can be managed if needed, by reducing the intensity of exciting light and by using more efficient ways of detecting emission. Thus, the common engineering issues raised against molecular computation are not problematic when the biological and chemical arena of molecular computation is acknowledged. Therefore, one of the original, albeit controversial, aims of nanotechnology[13] — to produce active molecular devices with information processing capability — is a realistic proposition.

a

Input (A)	Output			
0	0	1	1	0
1	1	0	1	0
Name	YES	NOT	PASS 1	PASS 0
Symbol				
Algebraic expression	A	$\overline{A}$	1	0

b

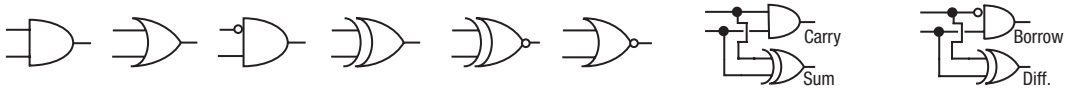
Input		Output						Output		Output	
(A)	(B)							(Carry)	(Sum)	(Borrow)	(Diff.)
0	0	0	0	0	0	1	1	0	0	0	0
0	1	0	1	1	1	0	0	0	1	1	1
1	0	0	1	0	1	0	0	0	1	0	1
1	1	1	1	0	0	1	0	1	0	0	0
Name		AND	OR	INH	XOR	XNOR	NOR	Half-adder		Half-subtractor	
Symbol											
Algebraic expression		$A \cdot B$	$A+B$	$\overline{A} \cdot B$	$A \cdot \overline{B}+\overline{A} \cdot B$	$\overline{A \cdot \overline{B}+\overline{A} \cdot B}$	$\overline{A+B}$				

Figure 2 Boolean binary logic operations: truth tables, symbols and algebraic expressions. **a**, 1-input logic. **b**, 2-input logic. The half-adder is composed of AND and XOR gates running in parallel to process the two input bits so that the AND gate outputs the carry digit and the XOR gate outputs the sum digit. The half-subtractor has parallel INH and XOR gates, which output the borrow and difference digits respectively. A 3-input full-adder is composed of one half-adder passing its sum digit to a second half-adder, with the two carry digits being admitted to an OR gate. A full-subtractor is constructed in an analogous manner.

MOLECULAR LOGIC

Logic operations can be achieved by using ions to control the fluorescence of a molecule[14]. In the example shown in Fig. 3, a molecule comprises three functional units separated by spacers. At one end of the molecule is an aromatic anthracenyl ring system (shown in blue) that would normally fluoresce blue (known as a fluorophore) when it is exposed to ultraviolet light. In this system, however, the fluorescence is quenched because of a faster process, known as photoinduced electron transfer (PET)[15], in which an electron is transferred (Fig. 3a) to the anthracenyl group from either the nitrogen or oxygen atoms in the other parts of the molecule. The nitrogen group can act as a receptor for hydrogen ions (that is, acids) and the ring of oxygen atoms can be used to capture sodium cations, which are just the right size to fit snugly inside. If one of these two receptor sites (shown in green) is occupied (Fig. 3b,c), PET will still occur from the other, and no fluorescence will be observed.

If, however, both receptor sites are filled (Fig 3d), that is, H^+ and Na^+ ions are both present, PET is prevented because the electrons are now tied up binding ions and strong fluorescence is observed. The overall result is AND logic, as an output of '1' (that is, bright fluorescence) only occurs when both ion inputs are present, that is, H^+ and Na^+ values of '1'. This general structure of fluorophore–$spacer_1$–$receptor_1$–$spacer_2$–$receptor_2$, in which PET pathways are either blocked or not, lies at the heart of many molecular logic systems. The properties of the fluorophore can be tuned as desired and each receptor can be modified to specifically recognize different guest species, in this case, G_1 and G_2, respectively. Other cases based on this molecular architecture — all with fluorescent PET parentage — will come up for discussion in the following paragraphs.

The recognition of logical content in photochemical phenomena has led to a myriad of related approaches, making molecular logic a truly general concept. Different systems can be classified according to several chemical or physical parameters (Fig. 1c). Various substrates, reagents, reaction conditions and readout modes have been commandeered. From a computing viewpoint it is clear that many kinds of inputs are used, each of which has its merits in a wider context. Biological entities contain many chemicals that can naturally serve as inputs to synthetic devices embedded therein. Electrical inputs to molecular logic gates can be supplied along wires by conventional semiconductor devices. On the other hand, optical inputs can be delivered remotely. Some of these cases featured in Fig. 1c need bulk materials, such as electrodes[8,16–19], gels[20–22], adsorptive media[23], stirred-flow reactors[24], or cuvet assemblies[25,26]. Although these approaches cannot be used for single-molecule detection, this level of sensitivity is not required for many studies and applications. In fact, only a few fluorescent logic gates have been examined at the single-molecule level[11] under carefully chosen conditions. The electrode-based examples have the advantage of being potentially in-line with semiconductor electronic technology[4]. Comprehensive collections of molecular

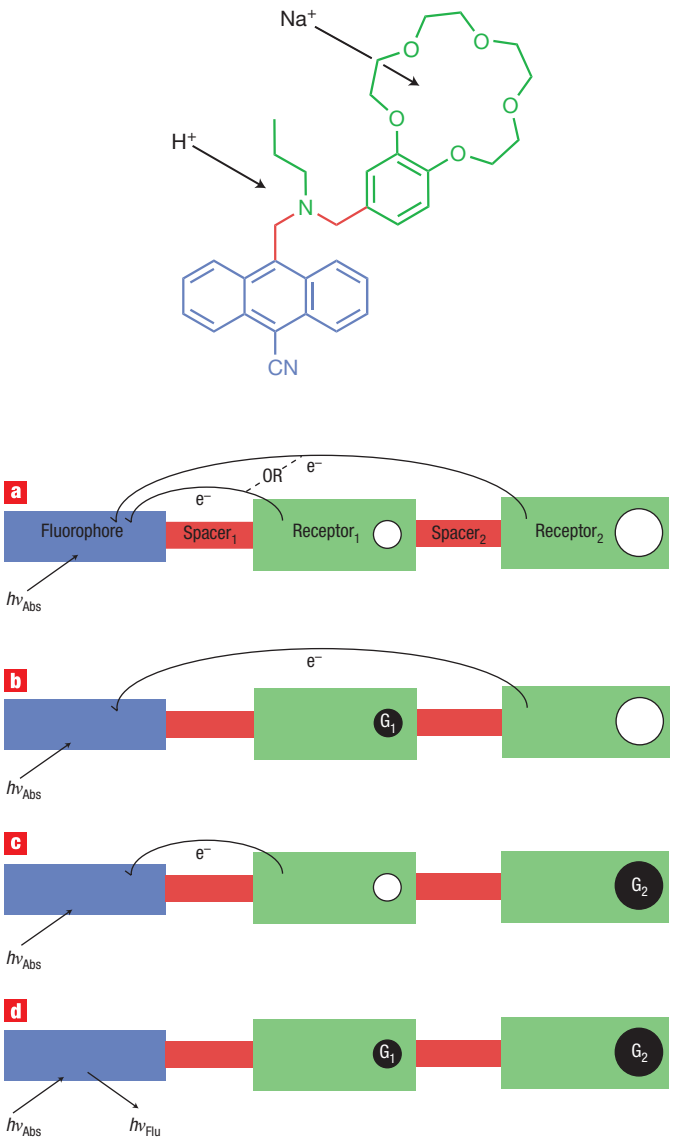


Figure 3 A molecular logic gate based on photoinduced electron transfer. Two chemical receptors (green) comprising an amine (receptor$_1$), which serves to bind H^+, and a benzocrown ether (receptor$_2$), which targets Na^+, are connected by hydrocarbon spacers (red) to each other and to a cyanoanthracene dye (blue) — a fluorophore that absorbs and emits light. **a**, The 'fluorophore–spacer$_1$–receptor$_1$–spacer$_2$–receptor$_2$' system has two possible paths of transferring an electron to the excited fluorophore — one from each unoccupied receptor — resulting in quenching of the fluorescence. **b**,**c**, Occupation of a receptor with its preferred guest cation blocks one pathway of electron transfer. **d**, Fluorescence results only if both receptors are occupied.

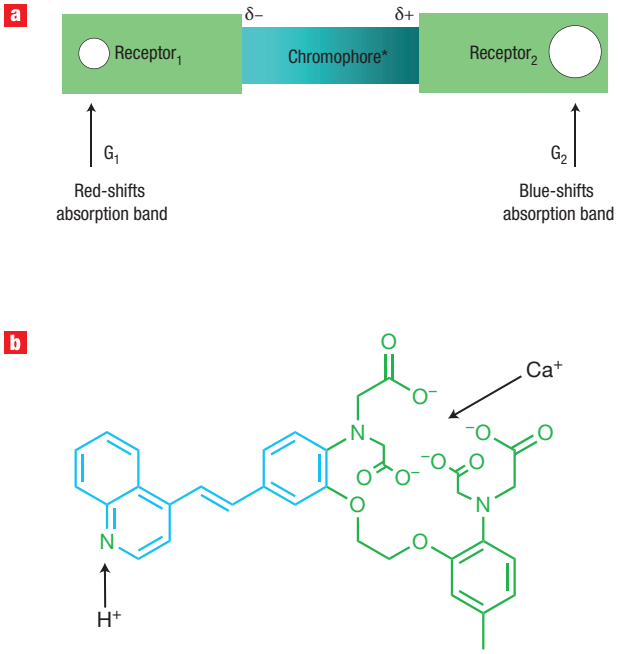


Figure 4 A molecular logic gate based on internal charge transfer. The photoactive component (chromophore) is shown in blue, and the chemically active components (receptors) are shown in green. **a**, When excited, the presence of electron-pushing and electron-pulling groups at the two ends of the 'receptor$_1$–chromophore–receptor$_2$' system causes a substantial shift of electron density from the electron-pushing group to the electron-pulling group. This causes the charge separation in the excited chromophore. When a cationic guest G_1 arrives at receptor$_1$, its proximity to the δ– charge causes a drop in the energy of the excited state, hence redshifting the absorption band. The opposite is true when cationic guest G_2 arrives at receptor$_2$ owing to the δ+ charge. **b**, The quinoline nitrogen (left) is receptor$_1$ and serves to bind H^+ as well as to be the electron-pulling group in the chromophore. The aniline nitrogen (right) is the electron-pushing group and is also a part of the Ca^{2+} binding amino acid receptor$_2$.

logic operations from the older literature are available[1,27–29] for the interested reader.

Although fluorescence is widely used as the output for molecular logic systems and can scale the heights of single-molecule detection, the simple effect of light absorbance should not be ignored. The ion-controlled colour change of 'receptor$_1$–chromophore–receptor$_2$' systems is illustrated in Fig. 4a and involves internal charge transfer (ICT) excited states[27]. These are electronic excited states with substantial charge separation, which arise through electron density moving from one end of the molecule to the other during excitation. The case in Fig. 4a is designed so that capture of the correct guest species by one of the receptors stabilizes the ICT state to produce a redshift in the UV–Vis absorption spectrum, whereas the other receptor/guest combination does the opposite. The design rests on the fact that receptor$_1$ is close to the negative terminal of the excited chromophore and that receptor$_2$ neighbours the positive terminal. Therefore, the presence of both guests (G_1 and G_2) leaves the absorption spectrum unchanged[30,31]. Hence, the case in Fig. 4b exemplifies this general approach to XNOR logic with absorbance output. The two amino acid units and the interspersed oxygen atoms form a three-dimensional crypt (righthand side of the green structure) of receptor$_2$, which selectively captures Ca^{2+}. The nitrogen atom within the quinoline group acts as the receptor$_1$ for H^+. As Fig. 2b shows, the output pattern of XNOR can be inverted to produce the pattern of XOR. As light absorbance is an inverse function of light transmittance, the case in Fig. 4b immediately becomes an XOR gate when transmittance is considered as the output.

As pointed out earlier, there are many properties that can be measured to determine the output of a molecular logic gate. In particular, monitoring changes in the rotation of the plane of polarized light in chiral molecules as a function of wavelength (a phenomenon known as optical rotatory dispersion or ORD) or in the differential absorption of circularly polarized light as a function of wavelength

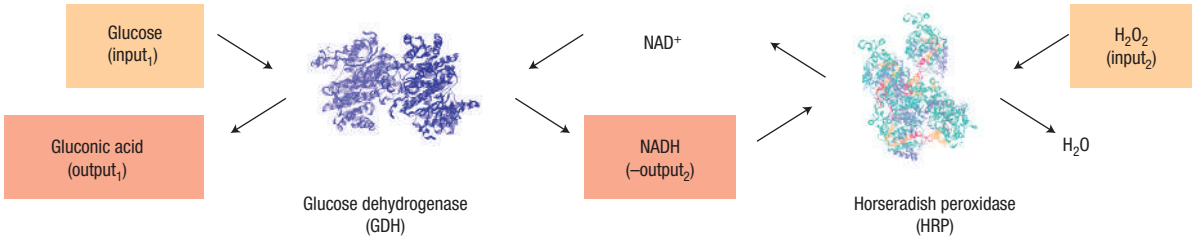


Figure 5 Molecular logic gates based on enzyme cascades. Coupling of enzymes by feeding the output of one as the input of another is a very useful way of developing molecular logic systems. While the enzymes are the devices that form the system, the various chemicals involved can be carefully declared as inputs and outputs. Starting conditions can also be set. For example, the fall in steady-state NADH concentration (output$_2$ '1') occurs if glucose is excluded (input$_1$ '0') from the enzyme system and H_2O_2 is admitted (input$_2$ '1'). This becomes INH logic (Fig. 2b). Adapted with permission from ref. 38. Copyright (2006) Wiley-VCH.

(known as circular dichroism or CD) can have advantages for reading the output. Whenever a molecular device is monitored by UV–Vis absorption spectroscopy or even fluorescence spectroscopy, excited states are invariably produced. As the energies of these excited states are comparable to the strengths of chemical bonds, this can lead to slow photochemical destruction even in the most carefully chosen cases, causing a limit on robustness. This has been considered under the 'Why molecules?' section, but laboratories keen on extending the useful readable life of molecular devices have turned to ORD and CD[32]. ORD spectra in particular occur outside the electronic absorption band as an absorption is a limited feature on the general dispersion stretching over large wavelength ranges. This is a reminder that many optical properties (for example, refractive index), unlike absorption, vary very gradually with wavelength. Thus ORD spectra can be monitored without populating excited states. As indicated above, ORD and CD properties only emerge from chiral molecules. Zhang and Zhu's work[32] on chiral photochromics can be enjoyed in this light because photochromics provide the switching ability that we require for logic operations. Indeed, photochromics can produce sharp changes in outputs such as light absorbance at a suitable wavelength when receiving light input at a different wavelength. More generally, inducing coloration of otherwise colourless photochromics with ions and light has examples spanning two decades[33–35] and has been developed more recently for logic purposes by Raymo and Giordani[36].

Biomolecule-based logic operations can be nicely illustrated with Willner's use of enzyme cascades[37,38] (Fig. 5). The enzyme glucose dehydrogenase (GDH) uses glucose as a substrate (input$_1$) in the presence of the cofactor NAD^+ to produce gluconic acid (output$_1$). Horseradish peroxidase (HRP) takes in H_2O_2 (input$_2$) to produce H_2O with the aid of NADH. These two enzymes run in parallel because the cofactor cycles between its two redox states (NAD^+ and NADH). If there is no NAD^+ initially, and if the system is allowed time to reach a steady state, the NADH concentration (as measured based on its absorption of light) drops only on the condition that glucose is absent and H_2O_2 is present. If this absorption change is taken as output$_2$, the coupled enzyme system manifests INH logic.

When glucose and H_2O_2 are both present, the NAD^+ produced by HRP's processing of H_2O_2 is taken up by GDH to process glucose so that the NADH level remains almost constant. On the other hand, gluconic acid (output$_1$, measured based on its absorption of light after reaction with hydroxylamine and ferric ion) accumulates only if glucose and H_2O_2 are both present, that is, AND logic is seen. If H_2O_2 is not available, NADH cannot be converted to NAD^+ by HRP so that GDH cannot process glucose in turn. Many native enzymes and cascades can be exploited, without any need for molecular synthesis in-house. Earlier enzyme-based logic by Conrad and Zauner was visionary but produced small signal changes[39]. Konermann's example also used a native protein (cytochrome c) to demonstrate AND logic[40]: if present together, urea and H^+ inputs reversibly denature the protein so that an amino acid (tryptophan) fluorophore avoids being quenched by a haem porphyrin, which is located at the protein active site. Another early case by Lotan[41], though requiring synthetic modification, has an appealing design. The active pocket of the common hydrolytic enzyme α-chymotrypsin is covered or exposed by an appended group whose geometry can be switched by photoirradiation. Proflavine, a classical drug, is an inhibitor of the enzyme, but if converted to its reduced form, the inhibitory power is lost. Naturally, the enzyme is active (output '1') only when its active site is exposed and when the inhibitor is powerless. This becomes AND logic if the coding of the two inputs (enzyme and the inhibitor) is chosen to fit.

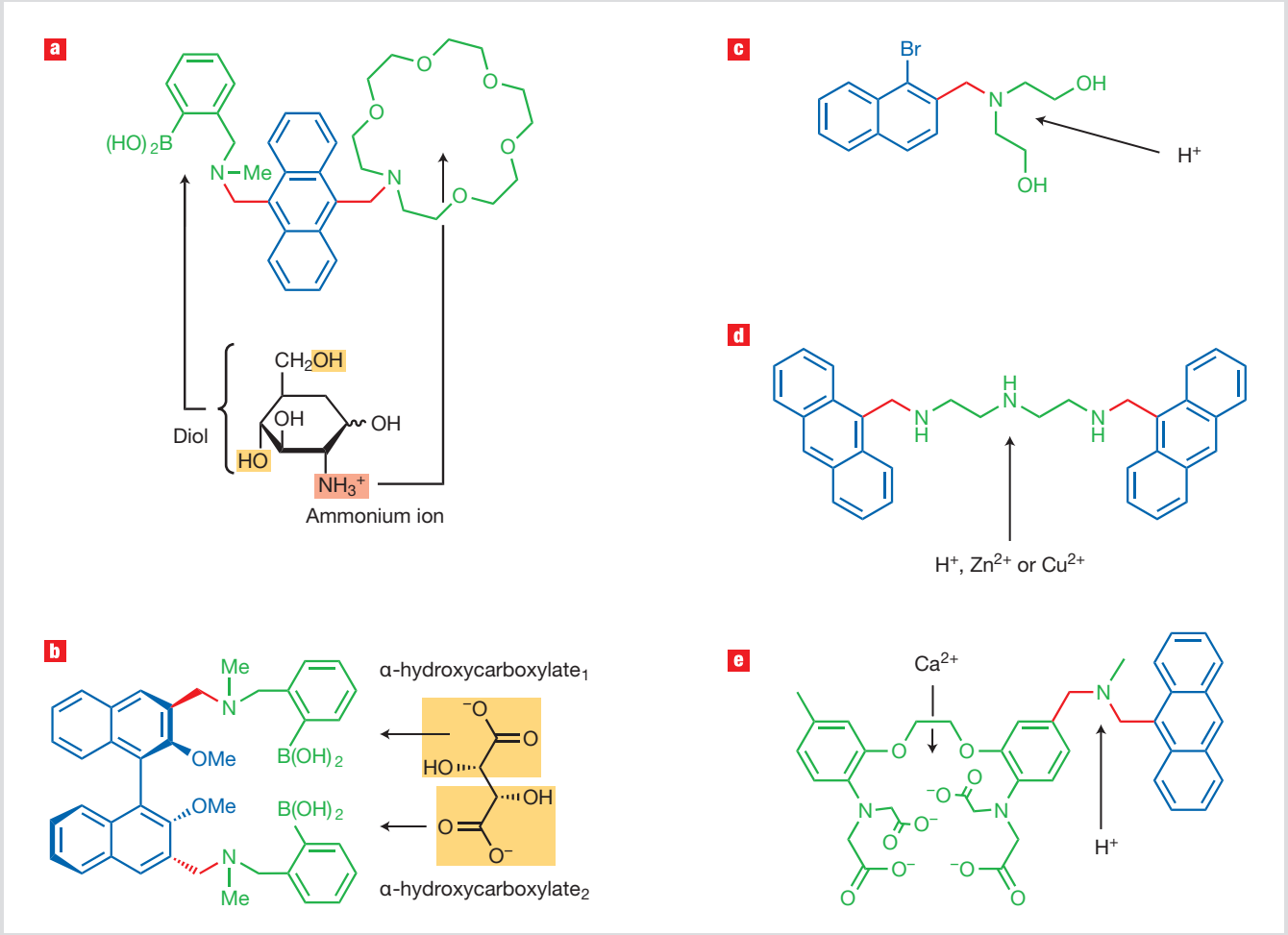
Besides enzymes, oligonucleotides have also provided lovely examples of logic. DNA-based computing, although without direct use of logic functions, has been known since 1994 (ref. 42). Stojanovic uses a DNA-based hydrolytic catalyst folded into a stem-loop shape where the catalytic activity resides[43]. An oligonucleotide with a weak link (at a ribonucleotide) is the substrate, designed to have good hydrogen-bonding complementarity with the ends of the stem-loop. The substrate carries a fluorescence energy donor and a fluorescence energy acceptor at its terminals so that we only see emission from the acceptor and not from the donor (output '0'). However, when the substrate is allowed to hybridize with the catalyst, hydrolysis occurs and the fluorescence energy donor is parted from the fluorescence acceptor. Then we only see emission from the fluorescence donor (output '1'). However, a new oligonucleotide (which will be our input) can be designed to hybridize with the catalyst by stretching it out, that is, the catalytic stem-loop is lost. Thus, when this input is present (input '1'), the substrate remains as it is and the fluorescence acceptor emission is seen (output '0'), that is, NOT logic.

We also note an interesting, though theoretical, proposal[44] to use magnetoresistance as a logic output from single molecules. Magnetoresistance is where an electrical resistance changes in response to a magnetic field, and is used in read heads of electronic computer hard drives. To extract this phenomenon from single molecules, cyclic π- electron systems connected to three metal contacts are proposed. The ring allows electron interference effects to take place. One contact carries the input electrical signal and the other two contacts serve to carry the two outputs in parallel. However, this requirement of three metal contacts to a molecule is difficult to realize at present[45].

LOGIC FOR SENSING

Sensing is an analogue experiment, with small changes of, say, fluorescence output arising from small changes of chemical analyte concentrations. Nevertheless, it is often exploited in an 'off–on' or digital manner for managerial 'go/no-go' decisions,

Box 1 Molecular logic gates based on PET



a, A selective sensor for glucosamine that binds to the diol (yellow) at one end and the ammonium group (pink) at the other. **b**, A selective sensor for D-tartaric acid, that contains two degenerate binding sites for oxygen-containing functional groups (yellow) at each end of the target molecule. **c**, A phosphorescence-based 3-input INH gate driven by H^+, β-cyclodextrin and O_2 inputs. O_2 is the disabling input. **d**, A logic gate with several logic configurations, depending on the inputs H^+, Zn^{2+} or Cu^{2+}. **e**, A H^+, Ca^{2+}-driven AND gate. The design of the sensors in **a**, **b** and **e** are similar to that of Fig. 3. **c** is similar, but simpler as only one receptor is involved. However a phosphor, rather than a fluorophore, is present. The design in **d** is similar to that in **c**, though an excited state interaction between the two fluorophores is an extra complexity.

particularly in medical diagnostic situations. This type of analysis corresponds to 1–input YES logic gates. The opposite type, with an 'on–off' response, is 1–input NOT logic. A few recent examples are noted here. Mice everywhere will sleep easier because of the development of an 'off–on' fluorescent PET sensor for saxitoxin[46]. Samples suspected of containing this marine toxin, originating in the red tide, were tested up until now with a mouse bioassay. Similar success has been achieved with PET sensors for monitoring cellular pH[47] as well as environmentally hazardous materials such as lead[48] and mercury[49]. A nice example of a fluorescent 'on-off' sensor for copper[50] has also been developed.

From a computational standpoint, chemically irreversible systems may not be all that appealing because they do not allow for a device to be reset — that is, once the output is set, it cannot be changed. In a broader context, however, such systems can be vitally important, especially for targets for which no receptors are known. In these cases, detection relies on the formation of chemical bonds — rather than a reversible binding event — and changes in the fluorescence of a given reagent are monitored. Among these difficult targets are chemical warfare agents such as diethylchlorophosphate. Two research groups applied fluorescent PET switching designs to this problem almost simultaneously in the same journal[51,52]. The first fluorescent PET reagents designed to produce irreversible 'switching' appeared in 1998 (ref. 53).

We have discussed AND logic gates driven by two separate chemical inputs (which can, in general, even be degenerate). Now, what if these two (or more) inputs were rigidly connected together? Whereas metal ions are not so convenient for this, many organic

functionalities can be easily joined-up in chosen geometries. For instance, even a simple sugar molecule is bristling with hydroxyl units rigidly arranged in three-dimensional space. This type of multifunctional molecule abounds in living systems. No wonder, as each of these is involved in the correct step of metabolic processes only when its specific receptor comes along. If specificity was lost, any other molecule within the compartment would be taken up by the receptor resulting in chaos within the cell. If we build molecular logic gates carrying multiple receptors, correctly positioned to temporarily capture these multifunctional molecular targets, we will have sensors with optimized selectivity, with much biorelevance. This avenue is so valuable that ideas of molecular logic and computation have a good future here, if nowhere else. We illustrate this with a fluorescent sensor selective for glucosamine[54] (See Box 1a). Old[55–57] and new[58] cases showing improved selectivity for diamines (either protonated or in their neutral state) with fluorescence/absorbance output are known. The fact that these diamines include the 'molecules of death' — cadaverine and putrescine — adds to the human interest of such sensing.

The ability to discriminate between different mirror-image forms of molecules (so-called enantiomers) can also be achieved with this approach. For example[59], when the chiral AND logic gate depicted in Box 1b binds to one enantiomer of tartaric acid (known as the 'D'-form), both receptor units are occupied and a fluoresence enhancement is observed. In molecular logic terms, both inputs are '1' and so there is an output of '1', manifested as an increase in fluorescence. On the other hand, L-tartaric acid, the mirror image form of D-tartaric acid, does not fit onto the AND gate as well and so one receptor site is not bound and PET prevents fluorescence. In this latter case, one of the inputs is '0' and, therefore, the output from this AND logic gate is also '0', that is, no fluorescence enhancement. This work is underpinned by earlier studies of this system[60,61].

INTEGRATION OF MOLECULAR LOGIC

The success of semiconductor-based computing depends on physical integration of unit logic operations. Much biological information processing also clearly depends on cascades down a stream. In contrast, the physical integration of molecular logic devices described so far is difficult. For instance, H^+ and Na^+ inputs produce fluorescence output from the AND gate in Fig. 3. It is not straightforward to take such a light output from one gate and convert it to an ionic signal to serve as input for a second gate. Indeed, the physical joining-up of many electronic devices succeeds because the same 0 V or 5 V electrical signal acts as the inputs and outputs in each of these devices. This is quantitative input–output homogeneity. In fact, the first molecular logic devices were unequivocally demonstrated because their inputs and outputs were so different that there was no danger of the output feeding back into the input channel and short-circuiting it.

When inputs and outputs are quantitatively homogeneous, they must be kept apart by arranging wired conduits for them. Such conduits naturally increase the complexity of the working device. In the case of metal wiring of molecular gates, controversies have arisen concerning the metal–molecule interface[62]. So it is no wonder that progress in molecular logic integration to produce small-scale gate arrays does not involve physical interconnection of molecular gates but rather uses ideas of functional integration outlined in 1999 (ref. 63), which removes the need for molecule–molecule linking. This means that the input–output pattern of a single molecular device operating via a set of mechanisms is recognized as fitting the truth table of a multigate array. For instance, the symbol for a NOR gate (Fig. 2b) shows how it can be physically constructed by connecting the input of a NOT gate to the output of an OR gate in an electrical context. On the other hand, the same truth table is achieved if we arrange for fluorescence quenching by two chemicals in a single molecular species[63]. Thus NOR logic with chemical inputs, as well as fluorescence output, is realized directly.

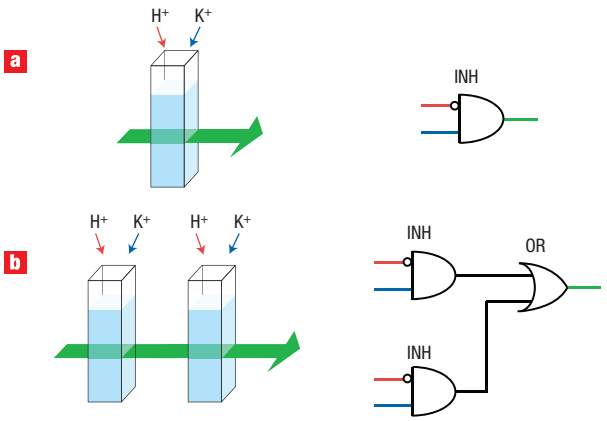


Figure 6 Integrated molecular logic gates based on colour-forming reactions. **a**, Sodium salt of $[Fe(CN)_5NO]^{2-}$ reacts rapidly with a thiol RSH to generate the purple-coloured $[Fe(CN)_5N(O)SR]^{3-}$ only if H^+ concentration (red input channel) is low and K^+ concentration (blue input channel) is high. The intensity of the purple colour is measured as the absorbance at 520 nm (green output channel). This is INH logic. **b**, Measuring the absorbance through two reaction cells in series naturally produces a result interpretable as a more complex logic gate array of two INH gates feeding their outputs into an OR gate.

Some recent examples of functional integration include 3-input INH[64], enabled NOR[65], and NOR[66] logic. The first of these, which is displayed in Box 1c[64], gives phosphorescence (a longer-lived version of fluorescence) as the output if H^+ and β-cyclodextrin are present and if O_2 is absent. The phosphorescence emission of the bromonaphthalene unit (shown in blue) is quenched by PET, as seen previously in Fig. 3 for fluorescence. The nitrogen atom serves as the electron donor. It also serves as a receptor for H^+, in which case PET is prevented allowing phosphorescence to reassert itself. However there are two more obstacles in its path. First, the excited state of bromonaphthalene can easily collide with another copy of itself to cause mutual annihilation. This deactivation process is prevented by enveloping the bromonaphthalene unit in β-cyclodextrin, which is a donut-shaped molecule with a sufficiently large cavity. Second, the excited state of bromonaphthalene is easily deactivated by collision with molecular oxygen. So, phosphorescence is observed only when molecular oxygen is removed from the sample. As is the norm in digital electronics[4], these cases are representative of arrays of component gates (2–input AND, 2–input OR and NOT).

Another innovation leads to higher levels of integration. Chemical experiments are often conducted in one pot. Additional complexity is naturally introduced by considering arrays of pots/cells. Up to now, we distinguished inputs by their nature (that is, H^+ or Na^+) alone. Now their spatial positions (or addresses) become available as a distinguishing feature. An increase in the number of inputs means that bigger truth tables will result. These are analyzable in terms of larger arrays of logic gate components. Raymo and Giordani were the first to illustrate this idea with acid-sensitive photochromics[25,36,67]. However, let's consider Szaciłowski's recent example[26] based on a classical colour test for sulphide that many of us encountered in school chemistry laboratories. Colourless $[Fe(CN)_5NO]^{2-}$ reacts with thiols — organic sulphur compounds of structure RSH where R is a general organic group — to produce purple-coloured $[Fe(CN)_5N(O)SR]^{3-}$, which absorbs 520 nm light strongly. So our output will be the absorbance

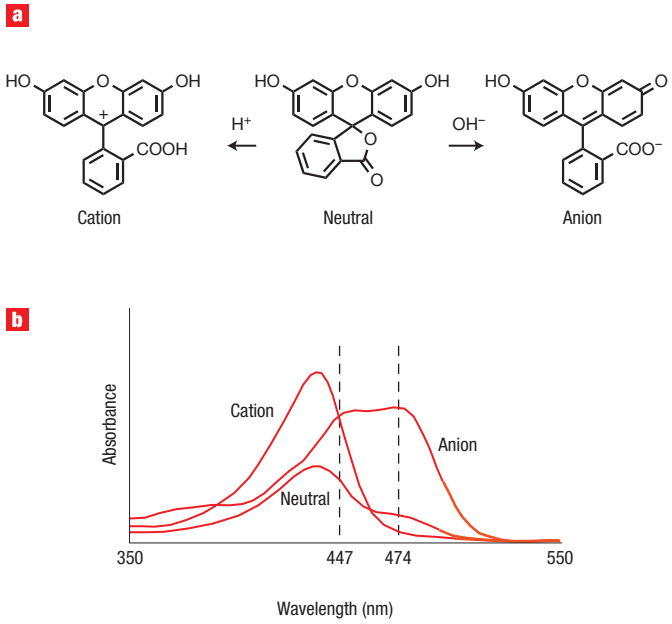


Figure 7 A moleculator based on fluorescein dye. **a**, Chemical structures of cationic, neutral, and anionic forms. Each of these can serve as the starting state of the molecular calculator, but we will focus on the neutral form (the middle structure). Equimolar doses of H^+ or OH^- are the chemical inputs applied. **b**, Electronic absorption spectra of fluorescein in water. Absorbance values at chosen wavelengths (for example, 447 and 474 nm) are the outputs. The relationships between these inputs and outputs can be cast into truth tables such as those in Fig. 2b. Reproduced, with permission, from ref. 78. Copyright (2006) ACS.

at 520 nm. Fig. 6a shows the zero-dimensional case. The reaction is run in a single cell. The product appears only if the concentration of H^+ is low and if the concentration of K^+ is high. K^+ stabilizes $[Fe(CN)_5N(O)SR]^{3-}$ by electrostatic attraction and drives the reaction forward. Low H^+ concentration is important for success because the reactive form of the thiol is RS^-. In high concentrations, H^+ will react with RS^- to produce electrically neutral RSH, which is less reactive. So the overall behaviour fits INH logic.

The one-dimensional variant is a line of two cells, with a 520 nm light beam traversing them (Fig. 6b). Obviously, a high absorbance is detected if either cell generates the product. As noted above, the condition for product formation is low H^+ concentration and high K^+ concentration. Both cells producing a purple colour would still give a high absorbance. Only if both cells have no reaction will we get a low absorbance signal. Thus the equivalent logic gate array becomes two INH gates feeding an OR gate (Fig. 6b). Szaciłowski extends this approach to two- and three-dimensional arrays of cells to strengthen his case. He also exploits the sensitivity of the reaction to many variables (nature of sulphide derivative, light, temperature, pressure and so on) to further increase its logical complexity, though not all variables produce non-linear behaviour.

RECONFIGURABLE MOLECULAR LOGIC

Up to now we have discussed molecules that were each designed to perform a given logic operation. Much of semiconductor-based computing hardware is also based on hard-wired logic circuits of a given configuration. The ability to change the configuration of a circuit on command is a more recent innovation of some power. This naturally allows the creation of multiple logic circuits, each optimized to carry out different tasks. Molecular logic gates can be reconfigured too. In fact, this can be done conveniently because, unlike electrons in semiconductors, many types of chemicals and many colours of light, which are all distinguishable from one another, serve as input/outputs[68]. The following paragraphs expand on this theme with specific examples.

The tried-and-tested path of fluorescence quenching by PET and its recovery by ion-binding is the basis of the action of di(anthrylmethyl)polyamine shown in Box 1d[69]. However this case differs from those discussed thus far by possessing two identical fluorescent anthracenyl units (shown in blue). When one of these is excited following light absorption, it can meet and 'stick' to the other within the excited state lifetime. The emission wavelength signature of the single anthracenyl unit is very different to that of the excited state of this dimer, which means that two fluorescence channels (at 416 nm and 520 nm respectively) can be observed. Each of these two emissions usually grows at the expense of the other so they produce different output patterns. For instance, the 520 nm emission dominates at low acidity, as the hydrophobic anthracenyl terminals crowd together in aqueous solution. The 416 nm band asserts itself at moderate acidity when the nitrogen atoms pick up protons, which cause a repulsion between the parts of the receptor chain (shown in green) and prevent the anthracenyl units from approaching each other. Thus the output at 416 nm indicates YES logic whereas 520 nm output displays NOT logic.

The logic behaviour of such PET-based anthrylmethylpolyamines is known to be reconfigurable according to the nature/level of the ion inputs[70]. Filled electron shell metal ions such as Zn^{2+} switch the fluorescence 'on' by blocking PET, whereas Cu^{2+} switches the emission 'off' (at a suitable pH). The latter effect arises because, at moderately high acidity, H^+ will bind to the nitrogen atoms, which will block PET and therefore switch fluorescence 'on'. But Cu^{2+} ions bind strongly to the nitrogen atoms, so when they are added, they drive the H^+ out. Unlike Zn^{2+}, Cu^{2+} is coloured (blue) — it has an excited state whose energy is lower than that of the anthracene excited state. Thus the fluorescence of the anthracene is switched 'off' by transfer of its energy to the Cu^{2+} centre. In general, the level of input rarely has a reconfiguring effect, but here the logic type can be altered by increasing the acidity — at high enough acidity, binding of Cu^{2+} is prevented and fluorescence quenching is not seen. Thus the acidity serves as a reconfiguring channel.

A subtler form of logic reconfiguring arises by changing the threshold of the 'high' or 'low' input signal. The fluorescent PET system shown in Box 1e[71] shows AND logic with H^+ and Ca^{2+} inputs provided that the H^+ input is defined as 'high' at pH 6. Alternatively, if the H^+ input is defined as 'high' at pH 2, the response converts to a simple PASS 0 gate where neither H^+ or Ca^{2+} inputs revive fluorescence. At such high acidities, Ca^{2+} cannot compete for its amino acid receptor owing to protonation. The availability of carboxylic acid groups under such conditions allows intramolecular hydrogen bonding with the π-electron system of the excited fluorophore (shown in blue), which drains its energy.

There are examples of reconfiguring molecular logic by means of the excitation/observation wavelength[30,36] in the case of ICT systems relying on analyte-induced spectral shifts (Fig. 4). A wavelength shift of an absorption or emission spectral peak naturally causes a rise in intensity at one wavelength and a fall in another. Then, as discussed under the monomer and excited dimer emissions of the case in Box 1d, different logic types arise when the intensities at two chosen wavelengths are monitored as outputs. It is quite easy to arrange for such monitoring to be done all at the same time. So, multiple logic configurations can be observed simultaneously in a single molecular species[30] — something that is difficult to achieve in simple semiconductor systems, but commonly considered in quantum computation, which places much store in simultaneous processing of

data. As ICT systems are the basis of molecular pH indicators found in pre-university chemistry laboratories and in flower pigments, the amazing conclusion is that aspects of quantum computers are present in your school and in your garden.

NUMBER HANDLING WITH MOLECULES

Number crunching is the public perception of computing, which any approach to molecular logic and computation needs to address. Molecular numerical operations have a variety of approaches[31,38,39,72–75]. The logic basis of numeracy has been expanded into gaming[76]. Remember your first sum? You learned that one and one is two. Implicitly you were introduced to the ascending hierarchy of numbers of zero, one and two. Importantly, your molecule-based brain learned this information for application throughout your life. A bit later, you were introduced to sum and carry digits. For example, when you tried to add seven to eight and ran out of fingers. Fifteen arose when one of tens was carried forward (to the left) and five of ones remained. The latter is the sum digit. Unlike decimal numbers from your childhood, the binary numbers in your calculator or computer deal with only zero and one, but the concept of sum and carry digits remains. The half-adder is the logic device which accomplishes this by running two binary digits into a parallel array of AND and XOR gates (Fig. 2b), where the carry digit is outputted from the AND gate and the sum digit emerges from the XOR gate.

Molecular-scale numeracy was first used in 2000 (ref. 31) when the 2-input AND gate (Box 1e) and the 2-input XOR gate (Fig. 4b), driven by H^+ and Ca^{2+}, ran in parallel. As in semiconductor-based half-adders, the AND gate output gave the carry bit while the XOR gate outputted the sum bit. Older cases of DNA molecule-based (but not molecular-scale, as gel electrophoresis is required) bit addition are known[20]. Wild's spectral hole-burning experiments for arithmetic are even older[77], but the logic operations were externally impressed on molecules rather than being inherent properties.

The recent moleculator (molecular calculator) from Margulies, Melman and Shanzer[78] is essentially pure and clever thought. Most of the previous experimental work was scattered in the literature, but Margulies *et al.* interpreted it from the vantage point of molecular logic. Their work revolves around the common fluorescent dye fluorescein, which can exist in several electrically charged forms depending on the pH value of the solution (Fig. 7a). It is shown how a full-adder and a full-subtractor (Fig. 2b) can exist within one molecular entity by integrating AND, XOR, INH and OR logic functions. The ability to observe different logic behaviours at different wavelengths (discussed in the section on reconfigurable molecular logic) and the use of inputs that are chemically identical but distinguishable to the experimentalist (known as degenerate inputs) are key to the success of this example. Let's sample this paper by discussing how the neutral state of fluorescein (the central molecule in Fig. 7a) can produce a half-subtractor (Fig. 2b). The basis of a half-adder was pointed out in the opening paragraph of this section. The half-subtractor is a similar arithmetic device, which permits subtraction of binary digits. It produces a difference digit and a borrow digit. These two are outputted by a XOR gate and an INH gate (Fig. 2b) respectively, operating in parallel.

The impact of H^+ ($input_1$) on the neutral state gives the cationic form whereas OH^- ($input_2$) gives the anionic form (Fig. 7a). Their absorption spectra (Fig. 7b) are clearly different from one another. This is an empirical observation. As discussed earlier concerning Fig. 4a, absorbance is a convenient logic output. Examining the spectra in Fig. 7b at 447 nm shows us that the absorbance is low (output '0') for the neutral form ($input_1$ '0', $input_2$ '0'). On the other hand, the absorbance is high (output '1') for the cation ($input_1$ '1', $input_2$ '0') or anion ($input_1$ '0', $input_2$ '1') form. We still need the fourth row of the right-hand column of the half-subtractor truth table (Fig. 2b). Under the present regime the input condition ($input_1$ '1', $input_2$ '1') means that the neutral form of fluorescein is plied with equimolar amounts of H^+ and OH^-. This causes mutual annihilation of the two inputs, or, in chemical terms, acid–base neutralization. So it produces the same output (output '0') as the first row ($input_1$ '0', $input_2$ '0'), that is, the absorption spectrum of the fluorescein neutral form. This is XOR logic.

A similar examination of the spectra in Fig. 7b, but at 474 nm, shows us that the absorbance is high (output '1') only in the anionic form ($input_1$ '0', $input_2$ '1'). All the other three sets of input conditions produce low absorbance (output '0'), which satisfies the truth table for INH logic, which is the left-hand column of the half-subtractor

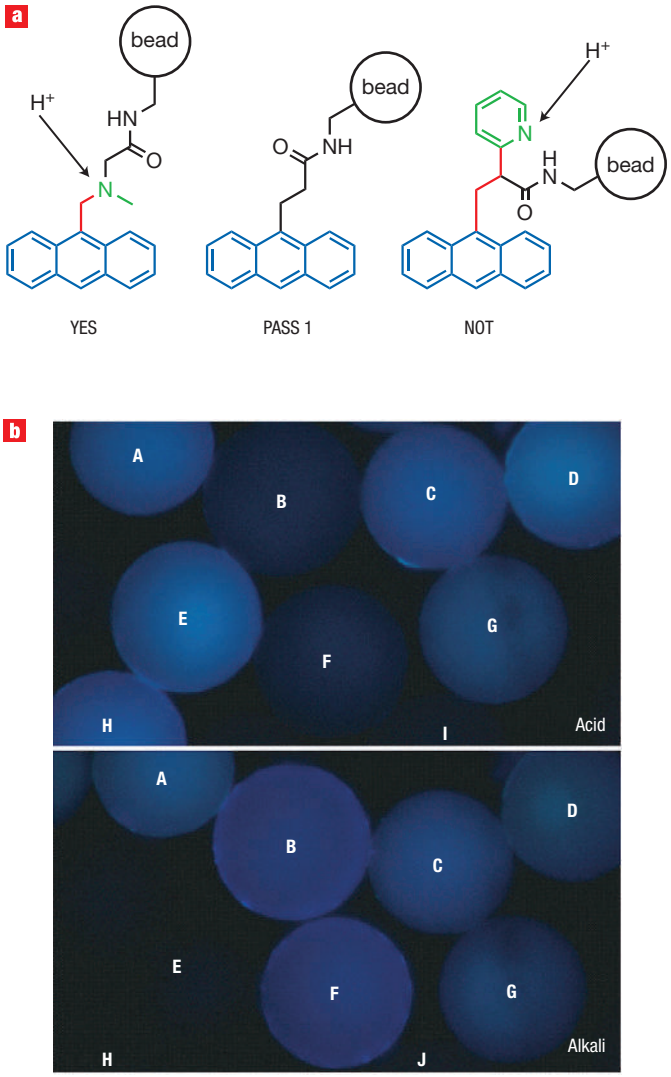


Figure 8 Molecular computational identification: materials and operation. **a**, Molecular YES, PASS 1, and NOT logic gates attached to small polymer beads. Their design is similar to that described in Fig. 3, with YES being simpler with only one receptor. PASS 1 does not possess a PET-active receptor. NOT has a receptor that permits PET only after guest binding. **b**, Fluorescence micrographs of beads with different logic tags. The H^+-driven logic type of each bead is assigned as A: PASS 1, B: NOT, C: PASS 1, D: PASS 1 + YES (1: 1), E: YES, F: NOT, G: PASS 1, H: YES, I: PASS 0. Condition: methanol:water (1:1, v/v) in the presence of acid (HCl) and alkali (NaOH) excited at 366 nm. Reproduced from ref. 89.

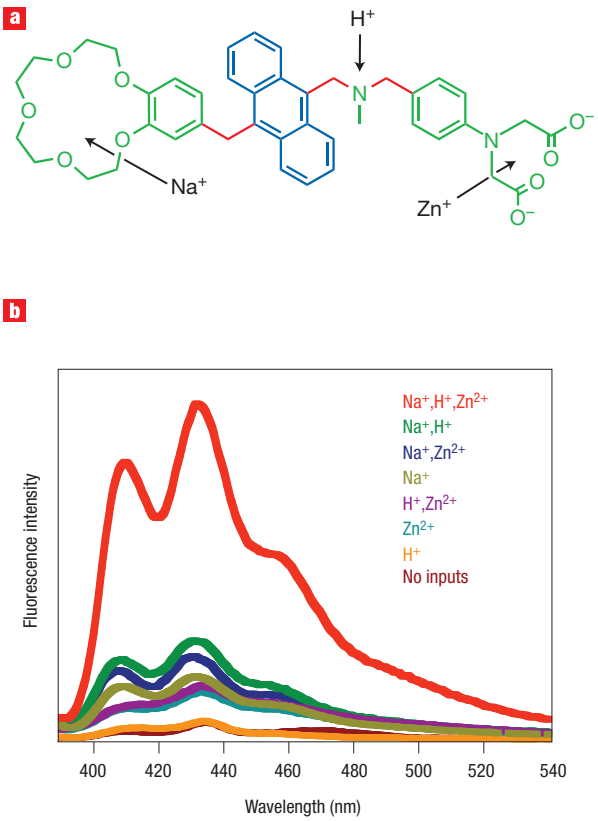


Figure 9 'Lab-on-a-molecule' and its operation. **a**, This design is similar to, but more complex than, that described in Fig. 3 as three receptors (green) are involved rather than two. **b**, Fluorescence emission spectra in water excited at 379 nm under the eight experimental conditions (colour coded). It is clear that the fluorescence is significantly stronger when the three inputs of Na^+, H^+ and Zn^{2+} are all present at sufficiently high concentrations rather than when only a pair, one or none of inputs are made available. Reproduced with permission from ref. 92. Copyright (2006) ACS.

truth table (Fig. 2b). The moleculator shows that a variety of selective receptors is not always required to demonstrate a rather complex logic array.

APPLICATIONS OF MOLECULAR LOGIC AND COMPUTATION

Molecular logic and computation has been around for 14 years and is beginning to come of age — the first applications of molecular computation are appearing. In fact, very sceptical opinions have been expressed[79,80] about this field. As a field develops, the question from sceptics changes from 'can it be done?' to 'can it do anything really useful?'. Then, new applications must address unanswered questions. Hearts and minds are won in general by applications of public interest and then a field can be said to have achieved some degree of credibility. Such applications are vital for the future of molecular logic and computation as the prognosis for solving real-world problems with DNA-based computation[42] is claimed to be poor[81].

Consider the general problem of object identification in populations. Cars have number-plates. People have faces/biometrics/passports. What of small objects in the micro/nanometric domain? These are crucial for (1) cells from patients being screened for diseases and (2) polymer bead supports used in combinatorial chemistry, which is the method of generating large families of related molecules by making many possible combinations of a given set of building blocks. Polymer beads allow the use of the clever 'split and mix' method of generating chemical diversity in a few steps[82,83]. Briefly, separate batches of beads are appended with building blocks, A, B and C, for instance. Then these batches are mixed well and split into three batches again. Treatment of the first batch with building block D, the second with E and the third with F produces beads built up with AD, BD and CD in the first batch, AE, BE and CE in the second and AF, BF and CF in the third. The procedure can be continued to build up chains.

Semiconductor-based object identification methods, that is, RFID (radiofrequency identification) are widespread for large objects[84], such as smart keys carried in your pocket or even your pet dog (once a suitable chip is implanted). However this approach cannot be applied conveniently to the small-scale situations mentioned above. Indeed, polymer beads used in combinatorial chemistry have been combined with RFID tags for commercial purposes only by pooling large numbers of chemically identical beads into a bigger container[85,86]. Coloured fluorescent dyes have been used to provide molecular tags for beads and cells[87,88]. However, molecular emission bands are quite broad in terms of their emission range. Therefore different bands tend to overlap, making it hard to distinguish many different tags or combinations of them, that is, fluorescent colour only allows us to conveniently distinguish limited numbers of tags.

A much greater number of tags can be distinguished if we add extra capabilities to fluorescent dyes by converting them into molecular computing elements[89]. First, computer elements have diverse logic types (Fig. 2). Second, unlike their semiconductor cousins, they have a large diversity of inputs: chemical species like H^+, Na^+, glucose and so on, and physical quantities like temperature, light dose and environmental polarity. Third, the input level, which triggers the output, also builds diversity. For chemical inputs, this threshold adjustment is made by tuning the corresponding receptor binding strength in the molecular gate. Fourth, and importantly, arraying the above gate choices to produce many-valued logic systems (known in computation[90] and indicated in chemistry[91]) gives a huge diversity running into many millions. In addition to these clear advantages for tagging, the method is simple to implement. The tagged beads, for instance, are gently washed with a solution containing a low level of the chemical input (input '0') and then their output fluorescence intensities are observed. The experiment is repeated, but after washing with a solution containing a high level of the chemical input (input '1'). This allows the build-up of a truth table from which the tag's logic identity is ascertained. The method is dubbed 'wash and watch' because of this washing of the beads followed by watching for the fluorescence output.

Fig. 8 shows two fluorescence micrographs obtained with 366 nm excitation. The beads in the sample are individually tagged with one of five H^+-driven logic gates. Four of these are YES, PASS 1, NOT and PASS 0 (Fig. 2a). The last means that the bead carries no tag and therefore emits no fluorescence (output '0'). The fifth is a logic array PASS 1 + YES (1:1), where precursors of these two logic gates are applied in equimolar amounts to derivatize one bead. A quick look is all that is needed to identify these. Let's do this identification one by one. Bead A glows blue with equally high intensity (output '1') following an acid wash (H^+ input '1') and following an alkali wash (H^+ input '0'). So bead A carries a PASS 1 logic tag. Beads C and G are more copies of beads tagged with PASS 1 gates. Bead B is virtually invisible (output '0') following an acid wash (input '1'), but comes alive with strong blue fluorescence (output '1') following an alkali wash (input '0'). Reference to Fig. 2a reminds us that NOT logic is the assignment. The same applies to bead F. Bead E (and H) is the opposite: glowing strongly blue (output '1') in acid solution (input '1'), but switching off (output '0') its fluorescence in alkali solution (input '0'). This is the YES logic (Fig. 2a) tag. Bead I remains switched off in its fluorescence (output '0') in both acid and alkali washes,

confirming its tag as PASS 0 (Fig. 2a). We kept the best for last: bead D. Fig. 8b clearly shows the significant fluorescence (output '1') in alkali solution (input '0'), whereas the fluorescence intensity doubles (output '2') following the wash (input '1'). Notably, this is not a binary 'on/off' situation but displays the two higher states of ternary logic ('2', '1' and '0'). This 2:1 output pattern can be seen to arise from the 1:1 sum of YES and PASS 1. So bead D is carrying the PASS 1 + YES (1:1). We note that all these logic gates use the same fluorophore, that is, tag diversity is seen even for a dye of one given colour. This method of molecular computational identification can be of immediate benefit in combinatorial chemistry where the use of large libraries based on polymer beads has been losing popularity.

Another area where molecular logic and computation can be applied is in intelligent diagnostics for medical purposes. We have already seen how molecular computing elements are contributing to improved sensing. Now we examine an extension of these. A patient visits a doctor. After a brief examination, blood tests are ordered. When the results are available, the doctor checks the series of parameters for those that overshoot the normal and those that undershoot. Then (s)he makes a logical combination of these outliers, in the Boolean sense, to lead to the diagnosis. For instance, a high cholesterol, a high LDL (low density lipoprotein) and a high CRP (C-reactive protein) indicates a cardiovascular problem. The typical fluorescence intensity–analyte concentration profile (response function) of a large population of identical copies of 'off–on' molecular sensors is S-shaped (or sigmoidal). Plateaux can be seen at very low and very high analyte concentrations for the 'off' and 'on' states respectively. The intermediate part of this response function is the sloping part where a small change in analyte concentration leads to a small change in fluorescence intensity, which is the sensing regime discussed in the section 'Logic for sensing'. We can assign the midpoint of the sloping part as the threshold where the 'off' state tips to the 'on' state and vice versa. The analyte concentration corresponding to this midpoint can be adjusted by controlling the analyte binding strength of the receptor unit within the sensor. We can choose/tune receptors so that only higher than normal concentrations of the analyte will elicit a fluorescence 'on' signal. By using several receptors within a multi-input AND gate, several 'high' analytes can be simultaneously detected with one fluorescence signal.

Fig. 9a shows a PET system[92] of the 'receptor$_1$–spacer$_1$–fluorophore–spacer$_2$–receptor$_2$–spacer$_3$–receptor$_3$' format which is a 3-input AND gate driven by H^+, Na^+ and Zn^{2+} inputs. Receptor$_1$ is the ring of oxygen atoms used to capture Na^+ and receptor$_2$ is based on the solo nitrogen used to bind H^+, as seen previously in Fig. 3. Receptor$_3$ is an amino acid derivative developed by Gunnlaugsson to catch Zn^{2+} rather selectively[93]. Each of these receptors can launch a PET process, in the spirit of Fig. 3 (except that the example in Fig. 3 contained two receptors instead of three), to the anthracenyl fluorophore. So each of these three receptors must be blocked by binding to its analyte present at above-threshold concentration for fluorescence to switch 'on' (Fig. 9b). This is a 'lab-on-a-molecule' as it performs three tests. It checks whether each parameter exceeds its pre-set threshold and then applies a 3-input AND logic function before releasing a 'high' fluorescence signal. Direct detection of analyte set logic patterns (cases for analyte pairs being known)[94,95] to produce 'well/ill' decisions[92] is another worthwhile capability of small molecules.

CONCLUSIONS

Designed supermolecules in the small nanometre range are highly functionalized nano-objects that can draw on two centuries of accumulated wisdom in chemistry. They display several foundational aspects of logic and computation, despite being a rudimentary mimicry of what traditional semiconductor logic does so well. All of the 1-input, 1-output and 2-input, 1-output logic operations have been demonstrated in the molecular regime. Many of these have been generalized to encompass various chemical species, absorption/emission colours and other variables, as well as various design principles. Small-scale integration, reconfigurability and numeric capability of molecular logic have all been shown. All of this proves that the field is growing well but it does not ensure its long-term vitality. This will come about when unsolved problems of a general nature are addressed. Luckily, this has now begun.

Molecular computational identification can identify small objects in large populations in biotechnologically important situations. Crucially, these objects are too small for traditional semiconductor logic to be applied. Molecular sensors based on logic ideas are already in service in intracellular situations where traditional semiconductor logic cannot go. Though not at the same stage of advancement, 'lab-on-a-molecule' systems combine sensing and logic to produce decision makers truly miniscule in size. However, the successes achieved so far are nothing compared with what is possible if fresh bright minds can be attracted into the field of molecular logic and computation. That is what this review hopes for.

doi:10.1038/nnano.2007.188

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