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Chemical fuels for molecular machinery

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Abstract: Chemical reaction networks that transform out-of-equilibrium 'fuel' to 'waste' are the engines that power the biomolecular machinery of the cell. Inspired by such systems, autonomous artificial molecular machinery is being developed that functions by catalysing the decomposition of chemical fuels, exploiting kinetic asymmetry to harness energy released from the fuel-to-waste reaction to drive non-equilibrium structures and dynamics. Different aspects of chemical fuels profoundly influence their ability to power molecular machines. Here we consider the structure and properties of the fuels biology has evolved and compare their features to those of the rudimentary synthetic chemical fuels that have been used to date to drive autonomous nonequilibrium molecular-level dynamics. We identify desirable, but context-specific, traits for chemical fuels together with challenges and opportunities for the design and invention of new chemical fuels to power synthetic molecular machinery and other nanoscale processes.

Main

Fuels are consumed to provide the energy that devices and processes require to perform useful work.¹⁻³ The free energy available from a chemical reaction can be harnessed by molecular machines and dissipated to offset work performed, thus preserving the Second Law of Thermodynamics when tasks are carried out through stochastic molecular-level dynamics.^{4,5} In this way chemical engines (Fig. 1)⁶ transduce energy from chemical fuels and have the potential to power synthetic molecular nanotechnology⁷⁻¹³ by driving and sustaining processes out-of-equilibrium.^{7-9,14} While some synthetic molecular machines use light,^{15,16} electrochemistry¹⁷ or transmembrane gradients,¹⁸ chemical fuels provide an attractive alternative energy source (Fig. 2).¹⁹ Although the waste generated in chemical fuel-to-waste reactions must be dealt with (either recycled, as happens with ADP in the cell, or removed, as occurs for water and CO₂ with aerobic respiration), chemical fuels are unencumbered by many of the issues faced by powering processes with other forms of energy. Photo- and electrochemistry often produce unstable intermediates, reactive radicals or cause photobleaching, all of which can limit the number of cycles that complex molecules survive for. Furthermore, in contrast to photons, chemical fuels have the potential to be stored and transported, enabling flexible and responsive systems that operate autonomously when and where they are needed.^{19,20} Indeed, active organisms that require a high-density energy supply (such as animals) tend to rely exclusively on chemical energy sources, whereas photosynthesising organisms cannot harvest enough energy from sunlight to support power-intensive behaviours, such as rapid movement. The same trend is apparent in technology; light-powered flight, for example, remains a major engineering challenge. For these and other reasons, while the energy input to biology largely originates from light (via photosynthesis), chemical fuels (such as adenosine triphosphate (ATP), Fig. 2A) are the primary mediators of energy used to maintain and sustain the complex nonequilibrium nanotechnology of the cell.^{4,20,21}

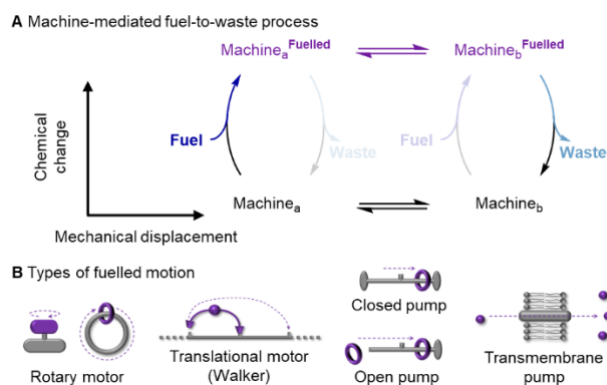


Fig. 1 | Chemically fuelled molecular dynamics. (A) The fuel-to-waste process mediated by the chemical engine cycle of an autonomous molecular machine.⁶ Autonomous molecular machines operate through information ratchet mechanisms.⁷ The fuel reacts preferentially with one mechanical state (differentiated by the subscripts a and b) of the machine: Machine_a reacts faster than Machine_b to form the fuelled state of the machine, Machine_a^{Fuelled}. A change in the mechanical state of the machine can occur in the fuelled state (Machine_a^{Fuelled} ⇌ Machine_b^{Fuelled}). The waste forming reaction occurs faster from the new mechanical state (Machine_b^{Fuelled} reacts faster to form waste than Machine_a^{Fuelled}). Mechanical interconversion of the unfuelled machine (Machine_a ⇌ Machine_b) completes the cycle. The machine is a catalyst for the fuel-to-waste reaction, while kinetic asymmetry²² generates directionally biased motion. Faded arrows indicate transitions that are slow relative to unfaded ones. To be consistent with microscopic reversibility, all transitions should be considered reversible. **(B)** Types of chemically fuelled molecular motion. Directional motion can be driven in rotary motors of covalently bound or mechanically interlocked species, translational motors (i.e. walkers), or in pumps. ‘Closed pumps’ (the substrate does not exchange with others in the bulk) use fuelling to generate a nonequilibrium substrate distribution while ‘open pumps’ (substrate can exchange with the bulk) sequester substrates from the environment to a collection thread or pump them across a membrane against a concentration gradient.

The term ‘chemical fuel’ has been used in the scientific literature in a variety of contexts, often without precise definition.² Drawing analogies between macroscopic and molecular-level phenomena is rarely perfect. Nevertheless, in this Review we find it helpful to use ‘chemical fuel’ to signify out-of-equilibrium reactants that provide the energy that is transduced (through kinetic asymmetry²²) by a chemical engine⁶ (a reaction network that converts energy from one form to another to perform a task²³ that is separate to, but accompanies, fuel-to-waste conversion, Fig. 1). ‘Waste’ refers to the products of the reaction as it progresses towards equilibrium (see Box 1). Which of the reactants/products actually corresponds to ‘fuel’ and which to ‘waste’ (i.e. the direction of the reaction supplying the energy that is transduced) can, in some circumstances, be reversed (a notable example is ATP synthase); but for any given task ‘fuel’ indicates the species consumed to power the chemical engine and ‘waste’ what is produced as a result. These definitions fit well with how these terms are generally used in a biochemical setting.²⁰ The properties and traits discussed in this Review apply particularly to chemical fuels capable of continuously supplying energy through information ratchet mechanisms⁷ (Fig. 2).^{1,2} Chemical reactions can also be used to transiently alter thermodynamic minima and kinetically trap responsive systems (for example, with pulse-fuelled (Fig. 5) or sequentially operated systems, Fig. 4A).^{2,3,24–27} Insights can also be gained from other stimuli-responsive supramolecular systems (Fig. 3), such as

dissipative self-assembly^{28–32} or micromotors³³ and other fuelled systems,^{34–37} even though kinetic asymmetry²² is not inherently necessary for their function.^{1,2}

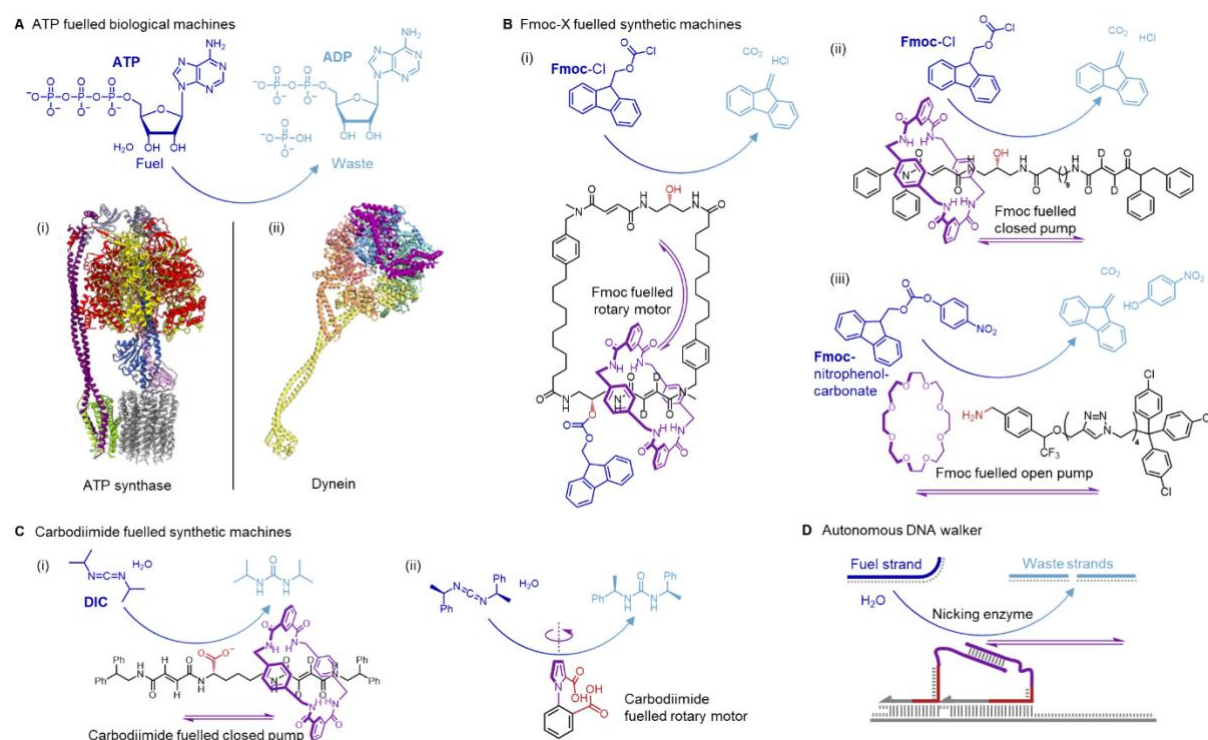


Fig. 2 | Chemical fuels used to power autonomous biological and synthetic molecular machinery. The machine catalysed fuel-to-waste reaction is indicated in blue. **(A)** Hydrolysis of adenosine triphosphate (ATP) powers numerous biological machines, including (i) F_1F_0 -ATP synthase,⁴ a motor/transmembrane pump, and (ii) dynein,²¹ a family of motor proteins that 'walk' along microtubule tracks. **(B)** Decomposition of activated Fmoc acyl derivatives has been used to drive (i) catenane-based rotary motors,¹⁰ (ii) rotaxane-based 'closed pumps'¹⁰ and (iii) 'open pumps'.¹¹ **(C)** Hydration of carbodiimides, such as diisopropylcarbodiimide (DIC), has been used to (i) drive the distribution of a macrocycle in a rotaxane-based closed pump away from equilibrium¹² (a process analogous to the Fmoc-Cl-fuelled closed pump in B(iii)¹⁰). (ii) Hydration of a chiral carbodiimide fuels directional rotation about a covalent single bond.¹³ **(D)** Enzymatic hydrolysis of a sequence-specific duplex can be used to fuel DNA walkers.⁴⁷ The catalytic site(s) of the machines are shown in red.

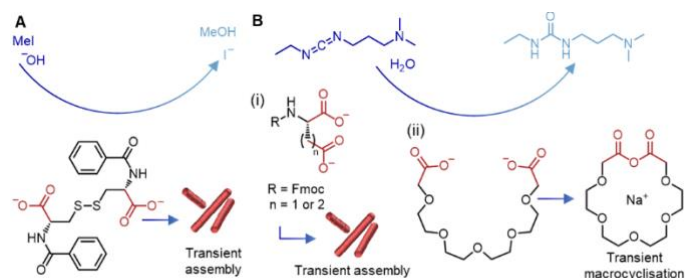


Fig. 3 | Chemical fuels for dissipative supramolecular assembly and macrocyclisation. Transient self-assembly of (supra)molecular structures using chemical fuels.^{28–32} Note: kinetic asymmetry is not an inherent requirement for such systems.² **(A)** Hydrolysis of MeI used to drive transient self-assembly of fibres.²⁸ **(B)** Hydration of carbodiimides^{29–32} used to power (i) the transient self-assembly of fibres²⁹ (ii) and the cyclisation of ion-binding macrocycles.³⁰ The catalytic site(s) of each system are shown in red.

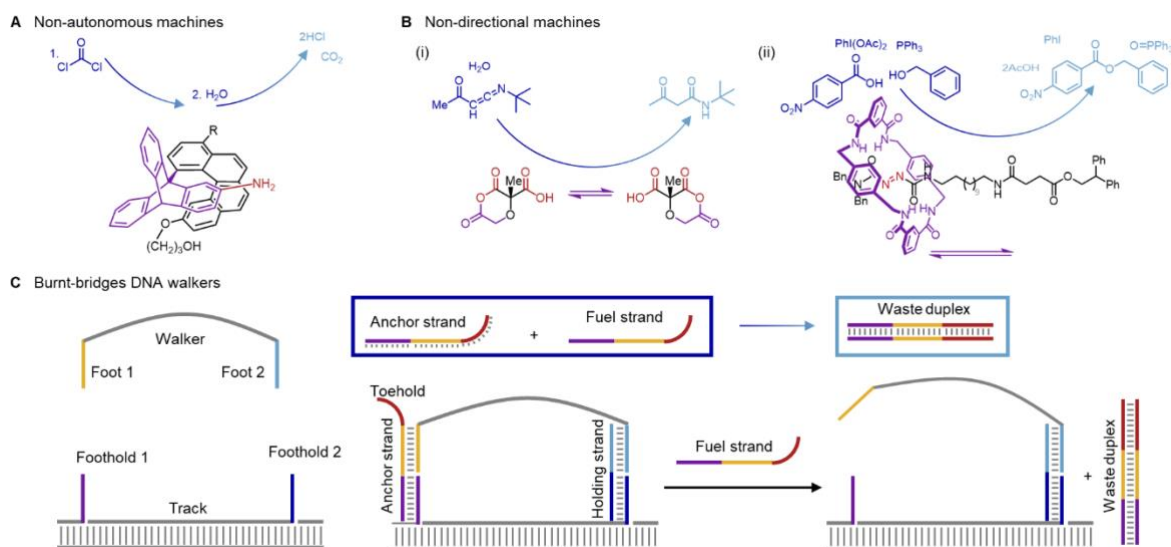


Fig. 4 | Chemical fuels for non-autonomous and non-directional molecular machinery. (A) Chemical fuels used in non-autonomous synthetic molecular machinery. Phosgene hydrolysis used to fuel a single 120° rotation in Kelly's molecular rotary motor prototype^{39,41} (phosgene and water are mutually incompatible and so must be added sequentially). (B) Chemically fuelled back-and-forth motion of molecular machines. These machines lack mechanical gating (Box 1) and hence cannot have directionally biased dynamics. (i) Hydrolysis of a ketenimine used by Mock to fuel intramolecular regiochemical exchange in a cyclic anhydride.⁴⁸ (ii) Mitsunobu reaction used by Berná to power shuttling within a rotaxane.⁴⁹ (C) Schematic representation of a typical burnt-bridges DNA walker system. Anchor and holding strands enable the walker to bind to track footholds by hybridization. Fuelling is driven by machine-mediated duplex formation between the fuel strand and the anchor strand. The catalytic site(s) of each system are shown in red.

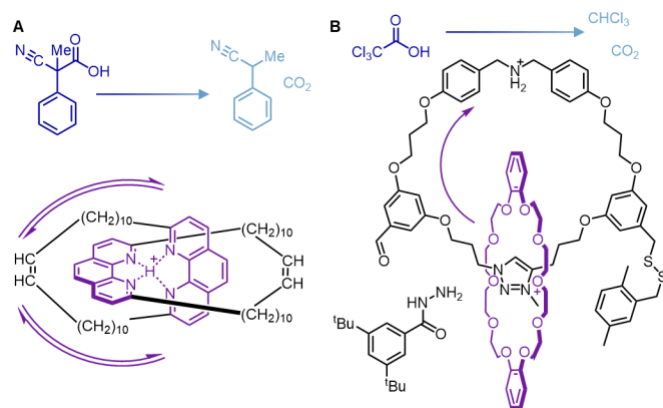


Fig. 5 | Molecular machines driven by chemical fuel pulses. Transient changes in the environment induced by a pulsed fuel can drive molecular machines. For such machines, the fuel-to-waste reaction does not need to be mediated by the machine (indicated by the straight arrows between reactant and products). **(A)** The decarboxylation of electron-poor carboxylic acids was employed by Di Stefano to generate transient acidic conditions that switch the co-conformation adopted by a [2]catenane.^{3,24,25} **(B)** Orthogonal dynamic covalent chemistry coupled to the decarboxylation of trichloroacetic acid was used to control directionality as well as the change in co-conformation, generating pulsed-fuelled rotary motors and open pumps.²⁶

Types of synthetic chemical fuels

To date, two main types of synthetic chemical fuel-to-waste reactions have been established to autonomously drive small-molecule machines: The decomposition of activated fluorenylmethyloxycarbonyl derivatives (Fmoc-Cl and Fmoc-nitrophenol-carbonate, Fig. 2B),^{10,11} and the hydration of carbodiimides, such as *N,N'*-diisopropylcarbodiimide (DIC, Fig. 2C)^{12,13}. The latter is one of a number of fuelled systems originally developed for dissipative assembly (Fig. 3).^{28–32} While the requirements for fuelling transient assemblies and directional dynamics differ, carbodiimide hydration has effectively transitioned between the two fields.^{12,13}

In early work, Kelly's exploration of rotary dynamics^{38,39} in a triptycene-helicene conjugate demonstrated the requirement, identified in Feynman's celebrated discussion of a miniature ratchet-and-pawl,⁴⁰ of an energy source to drive directionally biased rotation of a molecular-sized rotor. Kelly further developed this system to include a fuelling component (Fig. 4A),⁴¹ creating a rotor in which 120° directional rotation occurred upon reaction with phosgene to form an intramolecular carbamate. However, the fuelling process could not be made repetitious, in part probably because the reagents required to complete the cycle (through decomposition of the carbamate) react rapidly with phosgene such that the fuel-to-waste reaction is not appreciably promoted by the machine. The need to have the fuel react with the machine, and have that machine-fuel conjugate then form the waste and regenerate the machine while completing the mechanical cycle (rather than the fuel simply decomposing to the

waste through a background process not involving the machine), is a key problem in generating fuelling reaction cycles.

Single-use fuelling, which lacks a non-reciprocal pathway (i.e. the waste-forming pathway in Fig. 1) to regenerate the initial chemical state of the machine⁶ (such as Kelly's phosgene system), has been explored in a number of small-molecule 'burnt-bridges-like' machines (in which previously adopted (co-)conformations are rendered inaccessible),⁴²⁻⁴⁴ as well as being extensively used for driving synthetic DNA walkers.^{45,46} Many DNA walkers employ short oligonucleotide fuel strands that form hydrogen bonded duplexes through strand displacement reactions to drive the machines, rather than through the transient formation of covalent bonds (Fig. 4C). Sequence-specific enzymatic hydrolysis of DNA duplexes can also provide the fuelling input (Fig. 2D).⁴⁷ This process has an associated free energy release that can be used to drive nonequilibrium dynamics of the walkers.

An early example of an artificial chemically fuelled molecular system was provided by Mock (Fig. 4B(i)), who used the hydration of ketenimines to drive reciprocal regiochemical exchange in a small molecule.⁴⁸ While the lack of directionality (because of the absence of mechanical gating, see Box 1) precludes this system from operating as a motor, it provided a starting point for the subsequent development of the carbodiimide fuels originally introduced for dissipative self-assembly.^{12,29-31} Similarly, back-and-forth motion of a rotaxane shuttle was demonstrated by Berná (Fig. 4B(ii)),⁴⁹ driven by the catalytic formation of ester bonds by Mitsunobu reactions. Like the Mock system, this rotaxane lacks mechanical gating. However, the authors recognised the role of the machine as a catalyst and related aspects of the catalytic cycle to the mechanical state of the rotaxane shuttle.

The transient acidic conditions generated by the base-catalysed decarboxylation of electron-poor carboxylic acids has been used to switch the position of a macrocycle in mechanically interlocked molecules (Fig. 5A)^{24,25} and to directionally drive motors (Fig. 5B).²⁶ The operation of such motors requires a pulse of trichloroacetic acid for each 360° rotation or ring displacement to the next equivalent binding site. This inherently synchronises machine dynamics of an ensemble, a feature that may prove useful for correlating the motions within a group of molecular machines. This type of fuel is used in a very different manner to continuously acting (e.g. biochemical) fuels.¹⁻³ The pulsed acid fuel transiently changes the environment (and thus binding site preference and barrier lability), statistically biasing machine dynamics through an energy ratchet mechanism.⁷ In contrast to information ratchet

mechanisms, the interaction of the fuel with the machine does not give rise to kinetic asymmetry in the chemomechanical cycle (see Box 1).² Transient base conditions have also been generated with a pulsed-fuel²⁷ and have been used to temporarily switch the position of a macrocycle in a rotaxane, analogous to the process involving decarboxylative transient acids.^{24,25}

Autonomous chemically fuelled molecular motors

Autonomous synthetic chemically fuelled molecular machines (Fig. 2B–D^{10–12}) function through fundamentally the same type of information ratchet mechanism as their biological counterparts appear to.^{1,2,22} Consideration of the physics and physical chemistry of such mechanisms^{1,2,4,7,22,50} indicates that to generate directionally biased dynamics the following criteria must be met:

- The fuel-to-waste reaction must have a negative ΔG under the conditions of operation (i.e. be out of equilibrium), so that the free energy released can be harnessed by the machine.
- The fuel-to-waste reaction must induce directional bias through kinetic asymmetry²² in the reaction with the machine (e.g., through kinetic gating, see Box 1).
- The fuel must be kinetically stable, so that the fuel-to-waste reaction can be catalysed by the machine rather than proceed by an unproductive pathway.

In the subsequent sections we focus on the features of the fuel-to-waste reactions employed to date to autonomously drive synthetic molecular machinery: the decomposition of activated-Fmoc derivatives (Fig. 2B),^{10,11} the hydration of carbodiimides (Fig. 2C)¹² and the enzymatic hydrolysis of a DNA duplex (Fig. 2D).⁴⁷ We also consider what lessons can be learned from the chemical structure of biological fuels for the future development of synthetic fuels and machines.

Box 1 | Definitions of key concepts as used in this Review. Terms in bold are explicitly defined.

Term	Definition
Chemical fuel	The reactants in a fuel-to-waste reaction that release free energy to drive a nonequilibrium process, either continuously (via an information ratchet mechanism ⁷) or through pulsed or sequential operations (often via an energy ratchet mechanism ⁷).
Fuel-to-waste reaction	The exergonic conversion of a chemical fuel into waste products.
Fuelling reaction	The chemical reaction in the chemomechanical cycle where the fuel reacts with the machine to form a transient chemical state of the machine.
Waste-forming reaction	The chemical reaction in the chemomechanical cycle where the transient chemical state of the machine regenerates the initial machine state.
Chemomechanical cycle	Catalytic cycle for the fuel-to-waste reaction encompassing different chemical and mechanical states of the machine. ^{1,2,6}
Directionality	The overall ratio of forwards-to-backwards steps taken by the machine. Given as a ratio or percentage.
Kinetic gating	The kinetic bias to perform a forward process rather than its backward counterpart. Can be split into chemical gating and mechanical gating . Usually represented as a ratio of rates. In chemically driven systems this corresponds to the relative activation energies of the forward and backward processes.
Chemical gating	The kinetic gating of the chemical processes, determined by the mechanical state of the machine, that biases the fuelling and waste-forming reactions.
Mechanical gating	The kinetic gating of the mechanical processes, determined by the chemical state of the machine, that biases the direction of movement.
Kinetic asymmetry	The tendency of a chemomechanical cycle to progress in one direction over the other when driven by a fuel-to-waste reaction as a consequence of kinetic gating , quantitatively expressed ^{1,22} by the ratcheting constant (K_r).
Catalytic efficiency	The efficiency with which fuel reacts via a machine-catalysed pathway, rather than through a background reaction. Here we express it as the percentage of productive fuel use.
Thermodynamic efficiency	The proportion of available energy supplied by the fuel to the machine that is used to power directional motion and perform work. Does not include energy from the fuel that reacts via background processes. ^{5,50}
Overall efficiency	The product of the catalytic efficiency and thermodynamic efficiency as a proportion of the total energy released from the fuel-to-waste reaction .

Salient features of chemical fuels

ATP is a ubiquitous chemical fuel in biology, generally hydrolysed by biomolecular machines to generate adenosine diphosphate (ADP) and inorganic phosphate (Fig. 2A).^{4,20,51,52} At first glance its structural complexity is rather striking, as hydrolysis of almost any phosphate could, in principle, release similar amounts of free energy.⁵¹ However, the nucleoside (and length of the triphosphate region) serves as a recognition unit, allowing biocatalysts to bind this substrate specifically and hydrolyse a single phosphate unit (in some circumstances a second phosphate unit can also be hydrolysed).²⁰ The triphosphate group's charge reduces its susceptibility to direct hydrolysis.^{20,52} In addition to ATP, biology employs a range of other fuels to drive specific metabolic processes, such as guanosine triphosphate (GTP), creatine phosphate, carbamoyl phosphate, nicotinamide adenine dinucleotides (NADH/NADPH) and acetyl coenzyme A (acetyl-CoA).²⁰ This structural diversity allows fuels to be tailored for specific applications. Recognition elements enable fuels to be matched with the machines they drive so they can be targeted to specific processes without interference. For example, GTP orthogonally fuels microtubule assembly⁵³ that ATP-fuelled motor proteins (such as dynein, Fig. 2A) subsequently walk along.⁵⁴ Complementary base pairing likewise allows high specificity in the fuelling of artificial DNA-based machines.^{45–47,55} The incorporation of recognition motifs, and/or orthogonal chemistries, represents an opportunity to improve the performance and utility of the activated Fmoc^{10,11} and carbodiimide¹² fuels for synthetic molecular machines, which to date have lacked such structural elements. A recent carbodiimide fuelled rotary motor (Fig. 2C(ii)) uses a chiral fuel to generate kinetic asymmetry through diastereomeric interactions with the motor.¹³ Better expression of this diastereomeric relationship (by optimising the chirality of the fuel to the stereochemistry of the motor-molecule conformers) may improve the directionality of the fuelled operation of such machines.

Despite the structural diversity of biochemical fuels, a common feature is their requirement for additional reactants in the fuel-to-waste reaction;²⁰ biological fuels are reasonably kinetically stable and do not rapidly decompose under physiological conditions in the absence of a catalyst. This contrasts with the activated-Fmoc fuels in Fig. 2B that undergo background reactions that significantly compete with the machine pathway.^{10,11} Generally, hydrolysis or oxidation occurs in biochemical fuelling reactions, with water or oxygen effectively acting as a required second component of the fuel.²⁰ The abundance and availability of water and oxygen within cells is doubtless important for their role as partners in fuelling reactions, circumventing the need for additional complex organic molecular fuels.

This has been mirrored for artificial fuels in the reaction of carbodiimides with solvent water.^{12,29,30} Less intuitively, the track of DNA walkers is often a second reactive component of the fuel. Duplex formation between the fuel strand and track, or enzymatic hydrolysis of the track or fuel strand,⁴⁷ generally provides the free energy that drives these machines.^{45,46} In artificial systems the supply of reagents can be assured, providing the opportunity to explore a wide range of reagents, conditions and multi-component reactions.

Catalysis of the fuelling reaction

Autonomous molecular machines act as catalysts for the fuel-to-waste reaction and harness the energy it provides.^{1,2,12} Ideally, the background reaction rate should be zero, allowing the machine to use fuel at any rate without wasting energy stored in the fuel. However, this is not practical as all spontaneous reactions have an uncatalysed rate, however slow,⁵⁶ and it may be more difficult to optimise a machine to act as a good enough catalyst to utilise the energy from very slow processes when a fast response is needed. Instead, a balance needs to be found between catalytic efficiency (see Box 1)⁵⁰ and the overall rate of fuel use. ATP is only slowly hydrolysed under physiological conditions (at pH 8.4, 40 °C, $k_{\text{uncat}} = 3.2 \times 10^{-5} \text{ s}^{-1}$, half-life ~6 h),⁵⁷ so efficient catalysis by molecular machinery and controlled fuel supply enable >99.9% of fuel-to-waste reactions to proceed via machine-catalysed pathways.

Biology has evolved strategies to cope with the kinetic lability of fuels and optimise the balance between catalytic efficiency and rate of fuel consumption. Multiple levels of energy storage are used, each with different associated energies and rates for the fuel-to-waste reaction.²⁰ Particularly kinetically stable molecules, such as lipids and carbohydrates, provide a long-term energy store, but release large quantities of energy when oxidised.²⁰ However, these molecules are generally not suitable as fuels for directly driving molecular machines. Instead the energy is converted to a lower energy, more accessible and controllable form, often ATP.^{4,20} The kinetic stability of the fuel is generally matched with the process for which it is required; faster processes require less kinetically stable fuel molecules, such as creatine phosphate which fuels myosin motor proteins in muscles.²⁰ The relatively high background fuel-to-waste reactions of readily accessible fuels, such as ATP, render them unsuitable for storing energy for longer than a few minutes.

To maximise catalytic efficiency, the concentration of ATP in cells is generally kept relatively low (1–10 μM).⁵⁸ This concentration is maintained by replenishing the fuel, by synthesising it on demand.⁵⁹ Consequently, the background rate can be kept to a minimum and fuel is provided just fast enough to be used by the machine. Moreover, compartmentalisation means that conditions can be tuned and fuel localised to where it is required.^{60,61}

Through the use of high fidelity recognition motifs, DNA fuel strands can show excellent selectivity in reacting when DNA walkers reveal specific toeholds on the track,⁶² leading to low background rates of unproductive fuel decomposition.^{45,46} Alternatively, duplex formation between a fuel strand and a DNA machine can be used to activate a fuel to enzymatic hydrolysis⁴⁷ (Fig. 2D), a catalytic process reminiscent of the role of biological cofactors.²⁰ While synthetic small-molecule motors have not yet matched the sophistication of their biological counterparts as catalysts, parallels are beginning to emerge. The carbodiimide fuelled machines (Fig. 2C)¹² use a more kinetically stable fuel molecule (under machine operation conditions) than the Fmoc-Cl motor¹⁰ (Fig. 2B), and consequently up to 97% of the carbodiimide fuel reacts via the machine-catalysed pathway^{12,13} compared to <10% in the case of the Fmoc-Cl fuel.¹⁰ The lack of kinetic stability of the activated Fmoc fuels is an issue for the operation of both the activated Fmoc fuelled motor (Fig. 2B(i),(ii))¹⁰ and pump (Fig. 2B(iii)).¹¹ Changing from Fmoc-Cl to Fmoc-nitrophenol-carbonate improves catalytic efficiency, however the high background fuel-to-waste rate (in the presence of base) is best addressed by slow continuous addition of fuel into the reaction vessel, in analogy to the on-demand replenishing of biological fuels.^{59,63}

Chemical catalysis can be productively coupled with mechanical transitions to create a chemomechanical cycle.^{1,2,22} To maximize the effectiveness of such a cycle the mechanically exchangeable (co-)conformers should kinetically favour different transformations within the catalytic cycle so that the optimal catalytic pathway passes through all mechanical (co-)conformers.²² For example, one (co-)conformer may favour reacting with the fuel, while another may favour elimination of waste, generating kinetic asymmetry in the cycle (Fig. 1). Therefore, in order to most rapidly catalyse the fuel-to-waste reaction, the motor is driven between (co-)conformers,^{64,65} an action reminiscent of the challenges faced by switchable catalysts.⁶⁶ These pathways inherently generate chemical gating within a system and, coupled with appropriate mechanical gating, form the chemical engine⁶ that drives molecular motors (see definitions Box 1).

Design criteria for producing directional bias

Burnt bridges mechanisms, used by most DNA walkers,^{45,46} destroy the track behind the machine, preventing backward steps. However, directional motion can also be produced by non-destructive means by having the rates of the fuelling and waste-removal reactions vary according to the mechanical state of the machine (kinetic asymmetry).^{1,10–12,22,47} This occurs with the majority of biological motors that move along tracks^{4,21} and requires anisotropy in the machine structure.^{10,12,42}

For catenane and rotaxane-based small-molecule motors, anisotropy has been introduced by non-equidistant positioning of the catalytic site between macrocycle binding sites on the track.^{10,12} Biased displacement in the forward direction occurs by fuel addition being favoured when the macrocycle is far from the catalytic site (the distal position), the added fuel blocking subsequent backwards movement, and waste removal (re-allowing passage of the macrocycle) being favoured from a site proximal to the macrocycle. For the single bond rotary motor (Fig. 2C(ii)), anisotropy is generated through diastereomeric relationships between the rotamers of the machine and chiral reagents.¹³ Directionality arises from kinetic gating—that is the ratio of rates or the difference in transition state energies ($\Delta\Delta G^\ddagger$), of the fuelling and waste-forming reactions depending on the mechanical state of the machine, resulting in kinetic asymmetry.^{1,2,4,22} There is plenty of scope for the structures of both the fuel and the machine to significantly affect this ratio.

In biomolecular machinery ATP hydrolysis is catalysed in the binding pockets of highly specialised enzymes (Fig. 6A).⁶⁷ A range of structural features, including hydrogen bonding, metal ion binding and hydrophobic effects, contribute to stabilising the fuel-to-waste transition state(s).^{68,69} Consequently, biomolecular motors often operate with remarkable directionality (sometimes $\Delta\Delta G^\ddagger > 50 \text{ kJ mol}^{-1}$).⁴ In comparison the early generations of synthetic molecular motors are much less structurally sophisticated and effective. The Fmoc-Cl motor relies solely on steric hindrance between the fuel and macrocycle to kinetically gate fuel addition (Fig. 6B), while waste formation is unaffected by macrocycle position.¹⁰ This motor achieves a directionality of 4:1 ($\Delta\Delta G_{\text{fuelling}}^\ddagger + \Delta\Delta G_{\text{wasting}}^\ddagger = 3.4 \text{ kJ mol}^{-1}$). The DIC-fuelled rotaxane (Fig. 6D) also relies on sterics to gate fuel addition, but as the DIC fuel is rather small this generates a modest 2:1 ratio for the fuel addition kinetic gating.¹² However, the waste removal process is also kinetically gated in this system, apparently by hydrogen bonding between the macrocycle and the barrier stabilising the transition state for hydrolysis, resulting in a bias of 9:1 for barrier removal. These directional biases reinforce each other, resulting in an overall directionality of 18:1 ($\Delta\Delta G_{\text{fuelling}}^\ddagger +$

$\Delta\Delta G_{\text{wasting}}^\ddagger = 7.0 \text{ kJ mol}^{-1}$). This illustrates the effectiveness of kinetically gating both the fuel addition and waste removal steps and the value of designing interactions that stabilise transition states only in particular mechanical states of the machine.¹² The Fmoc-nitrophenol-carbonate powered pump (Fig. 2B(iii)) also gates both the fuelling and waste-forming processes. The transition state of the fuelling reaction is stabilised by the presence of the macrocycle, while the presence of the macrocycles sterically hinders the waste forming reaction (Fig. 6C). As the pump performs a different, if closely related, task (pumping on multiple rings rather than directionally displacing a single ring) direct comparisons with other rotaxane-based systems are not straightforward.¹¹ However, the result illustrates how it is the matching of characteristics of the fuel with the machine that determines the overall effectiveness, rather than it being an inherent property of either fuel or machine in isolation.

If other variables remain unchanged, increasing directional bias in a molecular machine should mean more efficient fuel use and a higher capacity to perform work. However, design efforts to improve kinetic asymmetry provide diminishing returns. Considering the relative rates/activation energies, an improvement in directional bias from 10:1 (~90%) to 100:1 (~99%) is more beneficial than an improvement from 100:1 (~99%) to 1000:1 (~99.9%), although each such order-of-magnitude improvement requires the same increase in kinetic asymmetry energy (a $\Delta\Delta G^\ddagger \sim 5.6 \text{ kJ mol}^{-1}$ at 20 °C). Eventually, the ever-decreasing benefits that come from increasing an already good directional bias are likely to be outweighed by other factors such as speed, catalytic activity and power output that may be adversely affected by optimising directionality.^{70,71} When designing chemically fuelled synthetic molecular machinery, the ideal balance depends on the task the machine is required to perform, as is also often the case in biology. For example, dynein is less well optimised for directionality than F_1F_0 -ATP synthase; both catalyse the hydrolysis of ATP to power directional movement, but dynein is optimised to work against an external load,^{5,70} quickly transporting cargos along tracks, whereas F_1F_0 -ATP synthase requires exceptional kinetic asymmetry in order to efficiently convert energy between a chemical store and a membrane gradient.⁴

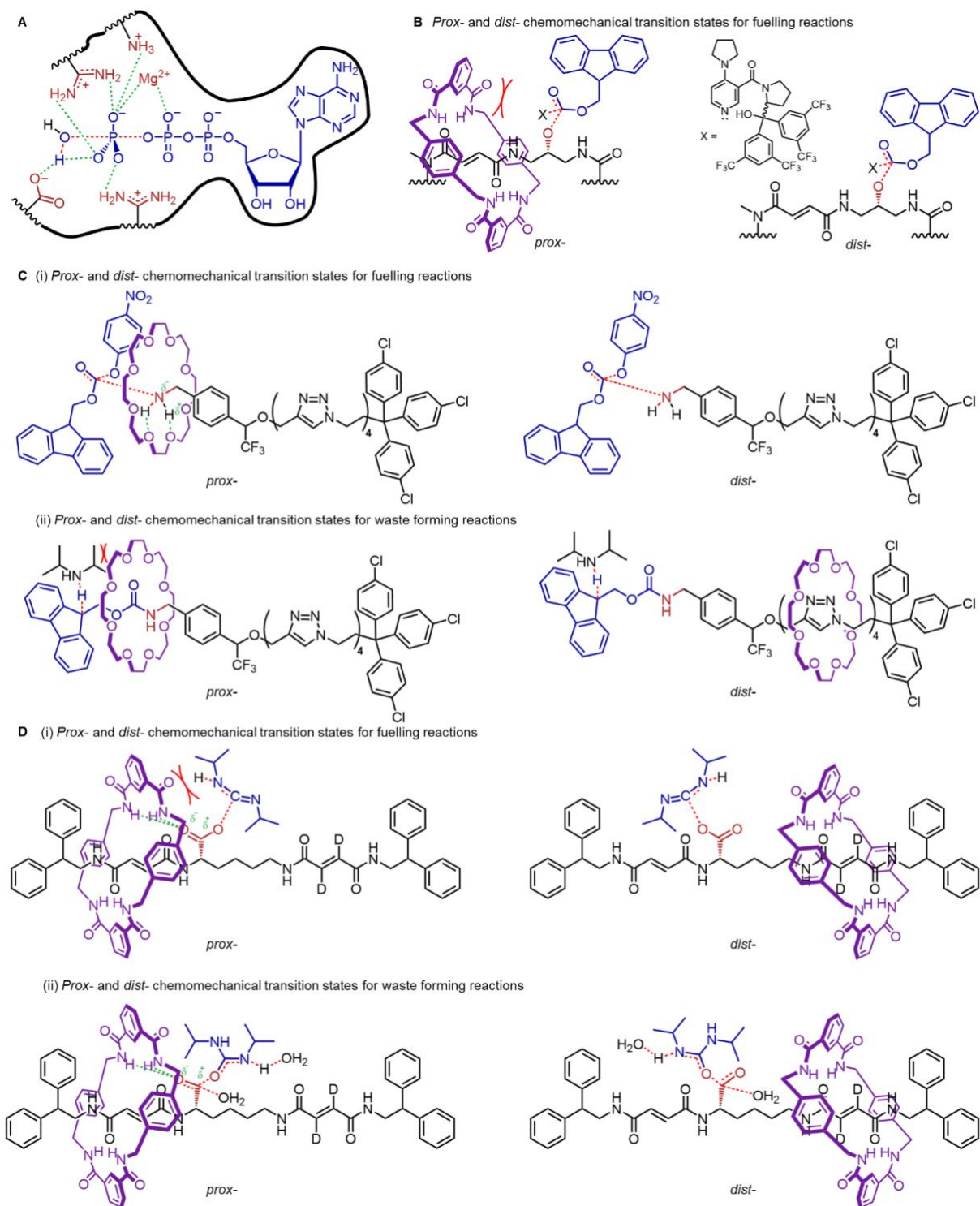


Fig. 6 | Transition states for fuel-to-waste reactions mediated by molecular machines. Formation/breaking of bonds in the transition state shown as dotted red lines. Stabilising interactions are shown as dotted green lines. **(A)** The binding of ATP within the binding pocket of an ATPase enzyme showing multiple stabilising interactions for the transition state.^{68,69} Chemomechanical transition states for **(B)** the Fmoc-Cl motor,¹⁰ **(C)** the Fmoc-nitrophenol-carbonate pump,¹¹ and **(D)** the DIC pump.¹² Steric hindrance favours fuel addition to the distal species for the Fmoc-Cl motor. This is similar for the DIC pump, though a stabilising interaction with the macrocycle also lowers the transition state energy for the waste forming reaction with the proximal isomer. Both fuelling and waste-formation are kinetically gated in the Fmoc-nitrophenol-carbonate pump by activation of the primary amine by presence of the macrocycle and steric deactivation of the carbamate to deprotection.

Energy content of the fuel

Given that its purpose is to supply free energy to a system, the fuel-to-waste reaction must proceed down a chemical potential gradient. Equilibration of the chemical potentials in the fuel-to-waste transformation releases energy that molecular machines make use of to perform work. In order to do more work, either more fuel—or a higher energy fuel-to-waste process—needs to be used.

The thermodynamic efficiency of a machine is the proportion of available energy supplied by the fuel to the motor that is used to power directional motion and perform work (not including fuel wasted in background processes), see Box 1.^{5,50,70} A machine that performs 20 kJ mol⁻¹ of work from a fuel-to-waste reaction that releases 100 kJ mol⁻¹ is less efficient (20%) than a machine that performs 50 kJ mol⁻¹ of work (50%) from the same fuelling process (by better kinetic gating). However, the thermodynamic efficiency would also be increased if the energy of the fuel-to-waste process was only 40 kJ mol⁻¹, while maintaining the work output at 20 kJ mol⁻¹ (50%). A task requiring the machine to perform less work than the energy released in a single fuel-to-waste reaction will intrinsically cause at least that excess of energy to be wasted. Accordingly, fuel-to-waste reactions that release relatively small amounts of energy are likely to be attractive for efficient molecular machines.

A widespread preference for low-energy fuel-to-waste processes is evident in biological machinery:²⁰ hydrolysis of phosphates releases only ~32 kJ mol⁻¹,⁵¹ while the dissipation of transmembrane concentration or potential gradients provides a precisely controlled release of free energy.⁴ Artificial DNA machines can also use low-energy fuel-to-waste processes; changing the length and sequence of an oligonucleotide provides a means of tuning the free energy release from the binding of DNA fuel strands.⁷² To perform high-energy tasks many biological processes employ multiple types and numbers of fuel molecules. For example, fatty acid synthesis derives energy from a variety of sources for the two-carbon extensions,²⁰ which may also increase efficiency.

Greater efficiency is not the only reason that low-energy fuel-to-waste processes can prove useful. A key benefit of low-energy fuelling processes is the opportunity for the molecular machine dynamics to be strongly coupled to the fuel-to-waste reaction, a condition that arises when the kinetic asymmetry ($\Delta\Delta G^\ddagger$) is greater than the free energy released from the fuel-to-waste reaction ($\Delta G_{\text{fuel-to-waste}}$).⁴ The ~32 kJ mol⁻¹ energy released by phosphate hydrolysis (such as for ATP)⁵¹ corresponds to a directionality of ~2.5×10⁵:1 at 37 °C (physiological temperature) to pass the strong coupling threshold.

Strong coupling has important consequences. Motors transduce chemical energy (from the fuel) to mechanical energy through movement. However, when a strongly coupled motor is forced backwards the mechanical energy can be converted into chemical energy as the motor takes the waste products and synthesises the fuel.⁴ Although macroscopically counterintuitive (a car does not make gasoline when pushed backwards!), this is a kinetically more probable pathway than the nanoscale machine moving without fuel or using fuel to move backwards. This is how F_1F_0 -ATP synthase manufactures ATP:⁴ a transmembrane proton gradient forces the strongly coupled ATP-driven motor in reverse, and in doing so drives the waste-to-fuel chemical reaction, synthesising ATP from ADP and inorganic phosphate.

Most biological molecular motors (kinesin, dynein, myosin, bacterial flagellum) are not strongly coupled.^{4,22,73,74} Instead, these weakly coupled motors appear to be optimised for other performance criteria rather than perfect directionality.^{5,70} These include maximising rate and efficiency while working against an external load, faster fuel use (and thus faster machine operation and task performance), decreased structural complexity, greater stability and greater flexibility. The complexity of biological systems makes it unclear which of these selection pressures are more important than others. This may become more apparent once synthetic fuel and molecular machine designs become more sophisticated.

Synthetic molecular machines are currently far from achieving the kinetic gating rate ratios found in biological systems (Table 1). Furthermore, the fuel-to-waste reactions currently used for synthetic molecular machines deliver a much higher free energy release than ATP hydrolysis. Hydration of carbodiimides has a free energy release of $\Delta G_{\text{fuel-to-waste}} \approx 80 \text{ kJ mol}^{-1}$,⁷⁵ while activated Fmoc decomposition is higher still ($\Delta G_{\text{fuel-to-waste}} \gg 100 \text{ kJ mol}^{-1}$), with a significant entropic contribution as a result of each fuel molecule being converted into three waste molecules. With these values, the strong coupling threshold is $\sim 2 \times 10^{14}:1$ for carbodiimides (at 20 °C) and $>7 \times 10^{17}:1$ for activated Fmoc fuels (at 20 °C). Since it is unlikely that such effective kinetic gating can be achieved, a more realistic option to improve thermodynamic efficiency and explore synthetic strongly coupled systems will be to develop lower energy fuel-to-waste reactions that match kinetic gating ratios that are more readily accessible for synthetic molecular machinery (Table 1).

Low energy fuel-to-waste reactions are not universally suitable for all tasks, however. Amongst these is energy storage, where it is desirable to use an energy-dense medium (such as fats and carbohydrates)

to store the greatest amount of energy for a given weight and volume.²⁰ Biology uses oxidation to release the energy stored in such molecules, although the large amount of energy these processes release makes them impractical for driving molecular machines directly.²⁰ The use of multiple energy storage molecules with different reactivity time scales and energy content is yet another useful trick of biology that synthetic molecular machines systems might do well to emulate. Storage of energy as a large reservoir of unreactive but high energy material, mediated by a more reactive intermediate that directly drives a machine, could be useful in improving the efficiency and viability of artificial molecular machinery.

Table 1 | Machine fuelling comparison of ATP with two fuels used to autonomously drive synthetic molecular motors (Fmoc-Cl¹⁰ and DIC^{12,13}) and enzymatic hydrolysis of a DNA fuel strand.⁴⁷

	Fuels			Oligonucleotide strand (+ H ₂ O)
	ATP (+ H ₂ O)	Fmoc-Cl	DIC (+ H ₂ O)	
Waste	ADP + Pi	dibenzofulvene + CO ₂ + HCl	Urea	Shorter oligonucleotides
Stoichiometry^a	2 → 2	1 → 3	2 → 1	2 → 2
Energy content^a /kJ mol⁻¹	32	>>100	80 ^b	22
Directionality^c	>>100000:1 ^d	4:1 ^e	18:1	30:1
Kinetic asymmetry /kJ mol⁻¹	>32 ^c	3.4 ^e	7.1	8.4
Catalytic efficiency	~100% ^c	<10% ^e	up to 97%	~9
Thermodynamic efficiency	40–100% ^f	<2% ^e	9%	~9

^a Of fuel-to-waste reaction

^b From DFT calculations⁷⁵

^c Estimated at 298 K

^d Estimated for F₁F₀-ATP synthase⁴

^e Determined for the Fmoc-Cl driven motor.¹⁰ The Fmoc-nitrophenol-carbonate driven pump¹¹ is not directly comparable as it performs a different, albeit related, task. However, the directionality of the Fmoc-Cl pump is significantly better than the Fmoc-nitrophenol-carbonate motor.

^f Varies depending on machine, maximum for F₁F₀-ATP synthase⁵⁰

⁹ Not reported.

Waste management

There are two types of waste that need to be considered for chemically fuelled molecular machines: waste chemicals and waste heat. Chemical waste products can disrupt a system by altering conditions

such as pH. For example, one of the three molecules that Fmoc-Cl releases in its waste-forming reaction is HCl (Fig. 2B), which requires neutralisation in order for the amine (required to decompose the Fmoc-ester in the fuelled state of the motor) to remain reactive. Alternatively, binding/interacting of waste with the machine can impede its operation through product inhibition or allosteric binding,⁷⁶ for instance, hydrolysed waste oligonucleotides can competitively bind to DNA machines.^{45–47} For low energy fuel-to-waste reactions, such as ATP hydrolysis,⁷⁷ the accumulation of waste can significantly reduce the chemical potential gradient for the fuel-to-waste reaction, which is the very energy source that the molecular machines harvest.

Biology has evolved sophisticated waste management solutions. One important strategy is the recycling of ADP and phosphate waste to regenerate ATP.⁵⁹ Consequently, ATP is a regenerable mediator of energy rather than a single-use fuel. This allows organisms to keep the quantity of ATP and ADP + inorganic phosphate far below what would be required without recycling.⁷⁸ Non-recyclable waste products are actively transported out of cells and circulatory systems in larger organisms to keep waste concentrations low. Similar effects have been achieved for synthetic systems, by using dialysis to remove the waste products of ATP hydrolysis, which acted as a stimulus to control supramolecular polymerisation,^{76,79} a strategy that may be extendable to fuel-driven artificial molecular machines. Currently, however, synthetic fuel systems generally have no sophisticated strategies for waste removal and rely on particular properties to limit the build-up of by-products, such as the insolubility of urea waste from carbodiimide fuels or the evolution of gaseous CO₂ waste from Fmoc fuels.⁷⁶ Appealingly, trichloroacetic acid, used as a pulsed-fuel to generate transient acid conditions (Fig. 5B), forms only chloroform (a solvent) and carbon dioxide (a gas) as waste products, neither of which would be expected to be detrimental to the operation of most molecular machines.

Waste heat generation from synthetic fuelling reactions provides both opportunities and challenges. Higher temperatures increase the rate of chemical and mechanical steps but may reduce selectivity and accelerate undesirable side reactions. Many biochemical systems are optimised to function at ~37 °C, probably balancing these two factors. Waste heat can be useful for maintaining ideal reaction conditions, as demonstrated by warm blooded animals that use ~60% of the waste heat from metabolic processes to maintain body temperature.^{70,80} Sophisticated thermostasis mechanisms have evolved to deal with residual waste heat.⁸¹ However, synthetic systems need not be restricted in the same way due to the availability of external cooling and heating methods. Sub-zero temperatures can be used

with operating environments other than water, potentially allowing improved directionality. Conversely, hotter temperatures could be employed for applications where speed rather than directionality is important.

Conclusions

There are two, far from mutually exclusive, ways to obtain an in-depth understanding of how to design machines. One is to discover or think up the necessary or relevant engineering principles from scratch. The second is to learn from the structures and mechanisms of working devices that already exist. With macroscopic machinery, early humans had no choice but to take the former approach and it took millennia to achieve the current levels of mastery over Newtonian mechanics. Unfortunately, these hard-won macroscopic engineering insights do not translate to machinery at the nanoscale. However, in biology we have an example of a working molecular nanotechnology and appreciating why different components have the characteristics, properties and functions they do can provide a short cut to understanding how (and, perhaps, why) to construct artificial machinery that functions at that length scale.⁸²

The chemical fuels developed to date for artificial molecular machinery are still rudimentary. A consideration of their mechanisms, along with the properties and roles of biological fuels, offers an opportunity to inform the design of new fuel molecules. A fuel mismatched to a machine or task will be wasteful if the fuel's rate of background decomposition is fast compared to the process it is powering, or if it does not induce a strong directional bias, or if it releases substantially more energy than the task requires. Synthetic fuels can potentially be tailored to specific task requirements^{20,70} or be appropriated from other fields that also use chemical engines, such as dissipative assembly (Fig. 3).^{2,3,6, 32,76, 83} The invention of low energy fuels for small-molecule machines would open the possibility of currently inaccessible behaviour brought about by strong coupling⁴. 'Designer fuels' with recognition elements incorporated into the fuel structure could likely improve both catalysis and kinetic gating.¹² Compartmentalisation^{58,84,85} would facilitate management of fuel activation, reaction concentration and waste removal,^{76,86} as well as providing more stable energy storage molecules to act as free-energy reservoirs. Many of these features appear to be within reach using established chemical concepts and methodologies. In the longer term, systems that use orthogonal or cooperative fuels may prove more

difficult to design,^{54,87} and sophisticated behaviour such as allosteric binding⁸⁸ might be necessary to fully emulate complex functions such as cell signalling pathways. Ultimately, fuel design represents an unexplored and fertile new opportunity to substantially improve the performance of synthetic molecular machines, and through which to develop, more generally, a rich variety of functions and applications for nonequilibrium (supra)molecular chemistry.

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