positive potential whereas the ion of lowest charge (Na^+) gave the least positive potential. This dependence on charge can be attributed to the Lewis acidity of the redox-inactive metal ions; the more highly charged Lewis acidic metal ions withdraw more electron density from the manganese centres, making them easier to reduce. Furthermore, the reduction potentials of the Mn_3X clusters showed a direct linear correlation to the pK_a value of a coordinated water molecule in the aqua complex of the redox-inactive metal ion. These values were used to quantify the Lewis acidity of the redox-inactive metal ions, and the correlation between pK_a and reduction potential shows that the influence of the redox-inactive metal ion can be directly attributed to its Lewis acidity. These results suggest that one possible role of the calcium ion in the OEC is to modulate the redox properties of the cluster; this subtle tuning of the cluster's redox properties is undoubtedly essential for the transfer of four electrons that is required for water oxidation.

Although the reduction potentials of the Mn_3X clusters correlate with the Lewis acidity of the fourth metal ion, X, one significant deviation from this trend cannot be ignored; the clusters containing Ca^{2+} and Sr^{2+} ions exhibit identical reduction potentials even though the Lewis acidities of the ions differ significantly. This result immediately calls to mind another important aspect of the OEC that is not understood: only a Sr^{2+} ion can replace Ca^{2+} in the active site and still maintain function of the enzyme, albeit with lower activity[2]. The connection of these two metals ions to the function of the OEC is unclear, but an emerging premise is that they have similar influences on the redox properties of transition metal complexes[4,8].

So, where do we now stand in our understanding of biological water oxidation? Agapie and co-workers have developed methods for preparing a series of structurally similar heterometallic clusters in which the identity of one metal ion in the cluster can be changed, leading to a proposed role of the calcium ion as a modulator of the redox potential in the OEC cluster. Despite this advance and numerous other synthetic and biological studies in this field, we still do not fully understand the mechanistic steps that lead to the conversion of water to dioxygen. Synthetically, an obvious next challenge is to incorporate function into these systems, which is likely to require the addition of another manganese ion to model the OEC in its entirety. These types of system are needed to augment the biophysical and biochemical studies on the OEC that will describe the relationship between the metal cluster and the protein matrix, which seems to be essential for efficient function. It is through this kind of interdisciplinary and integrated study that a full understanding of water oxidation will be realized. ❐

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INTERLOCKED MOLECULES

A molecular production line

A small molecule that mimics the sequence-specific peptide synthesis of nature's ribosomes paves the way for more elaborate artificial molecular synthesizers.

Paul R. McGonigal and J. Fraser Stoddart

Molecular machines of staggering complexity are employed routinely in biological systems to perform all manner of tasks that are crucial to sustaining life. Although chemists have begun to create small molecules capable of mimicking the mechanisms by which nature's motor molecules are driven[1–4], artificial molecular machines (AMMs) remain rudimentary compared with their biological counterparts. Writing in *Science*, David Leigh and co-workers[5] now present a significant step forward in the pursuit of AMMs capable of performing complex tasks, in the form of a wholly synthetic small molecule that functions as a pared-down version of nature's ribosome and constructs an oligopeptide from amino acid building blocks.

Ribsomes play a key role in decoding and replicating the information contained within DNA strands, by reading the blueprints present in the nucleotide sequences of messenger RNA, and fusing together amino acids supplied by transfer RNAs in a prescribed sequence[6–8]. Each ribosome is a self-assembled conglomerate of several RNA and protein chains, dwarfing Leigh's artificial peptide-synthesizing machine, which is approximately one thousandth the size. It is a testament to the ingenious design of the AMM reported in this study that, despite its diminutive stature, it performs a task akin to the sequence-specific polymer synthesis that occurs in living organisms. The structure of the AMM is based on a rotaxane architecture — a ring trapped on a dumbbell component by insurmountable steric barriers in either direction (Fig. 1a).

With three amino acid residues acting one after the other as barriers, and a tripeptide appended to the ring, the AMM is primed to synthesize a hexapeptide derivative in a pre-programmed sequence (Fig. 1b). Inclusion of a cysteine residue as part of the tripeptide attached to the ring is a critical design element, and the machine is 'switched on' once its reactive thiolate is unmasked under basic conditions. Each amino acid residue is liberated from the rod and transferred to the N-terminus of the peptide chain by native chemical ligation[9]. The labile phenolic esters that tether the residues to the dumbbell are attacked by the thiolate and undergo a transacylation reaction, before *S*-to-*N* acyl transfer moves the amino acid to the end of the growing peptide chain. In the process of picking up amino acids, the ring unblocks its own path along the dumbbell until it is able to slip off the end.

An impressive feature of Leigh's AMM is that no sequence isomers or other peptide by-products were observed, indicating that the machine is processive (that is, it cannot

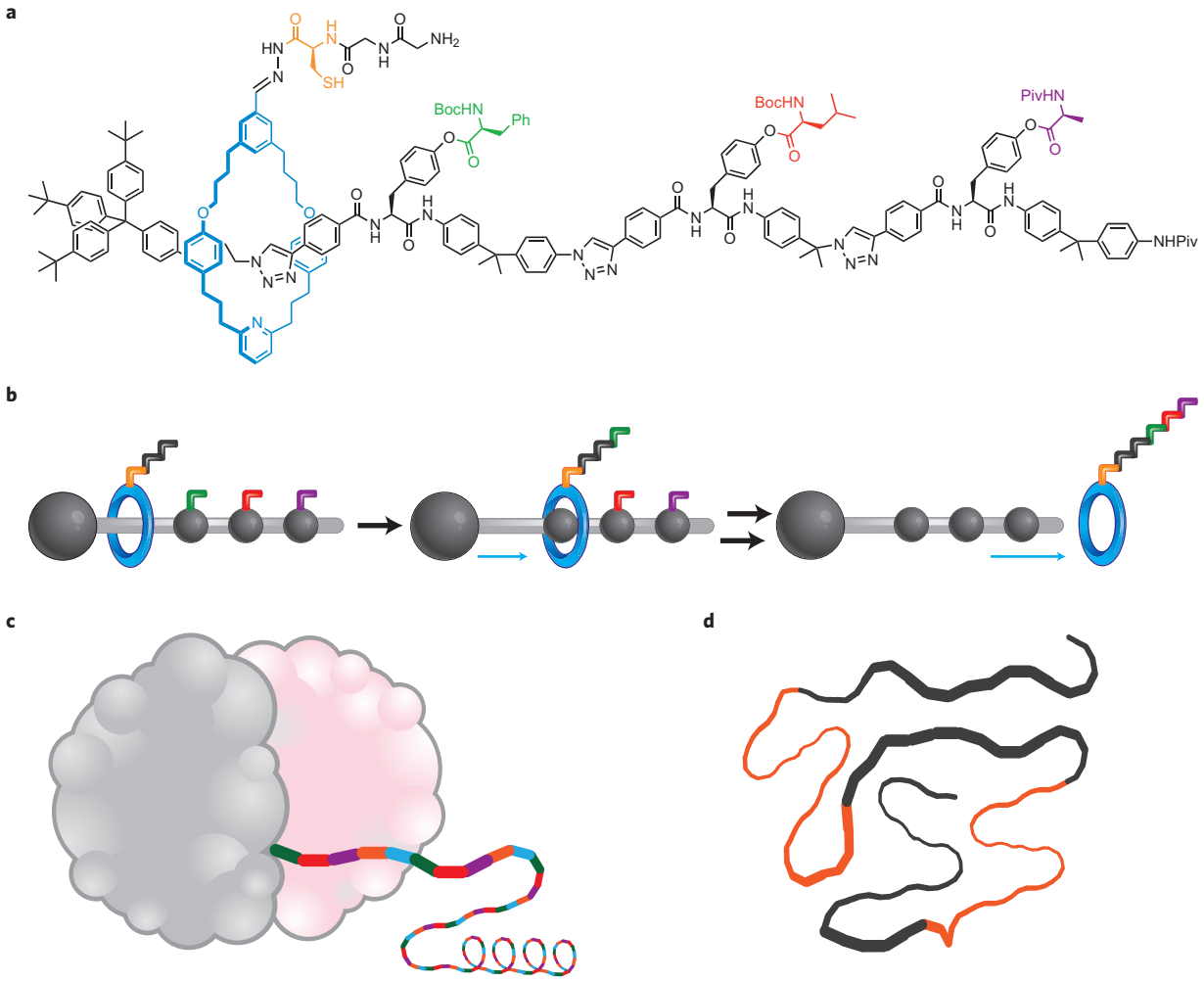

Figure 1 | Molecular machines. **a**, An artificial molecular machine that makes an oligopeptide with a pre-programmed sequence of amino acid residues. **b**, As the ring component (blue) moves along the rod (grey), a reactive thiolate (orange) picks up the amino acids (green, red and purple) and attaches them to the end of a growing peptide chain. **c**, The ribosome constructs proteins with precise control over the sequence of amino acid residues, which may then fold into a prescribed three-dimensional architecture (for example, a helix). **d**, Man-made polymers are, by comparison, unsophisticated and lack well-defined shapes for the most part. Boc, *t*-butyloxycarbonyl; Piv, pivaloyl.

skip over an intact barrier into another compartment of the rod) and operates with high fidelity for the encoded information. Error-free translation, however, becomes much more challenging if this system is elongated because the *S*-to-*N* acyl transfer step will be progressively less efficient as the peptide length increases. This limitation restricts the theoretical output of this first-generation AMM to oligopeptides of up to roughly 10 amino acids in length. Nevertheless, the rotaxane architecture used in this research provides a convenient way with which to trap the two components of the AMM kinetically, while enabling relatively unrestricted large amplitude movement of the parts relative to one another. Future AMMs that perform chemical synthesis need not, however, be limited to mechanically interlocked molecules.

Parallels can be drawn between the mode of action of this AMM and the biological machinery it imitates (for example, the interlocking of the ring around the rod can be likened to the way in which messenger RNA is clamped by the ribosome); yet, the differences between the natural and artificial systems also warrant consideration. Ribosomes utilize energy supplied by the hydrolysis of adenosine triphosphate (ATP) each time an amino acid is attached to the end of the nascent protein strand, whereas there is no need to couple consumption of a fuel to the operation of the AMM in this present study. Amide formation at the expense of phenolic esters is enthalpically favourable and provides the major thermodynamic driving force for the reaction cascade, while thermal noise is responsible for creating movement of the ring in relation to the dumbbell. Deprotonation of the thiol is all that is required to start the machine; it operates autonomously without any further intervention.

In essence, the machine is constructed in a high-energy state and moves energetically downhill in steps once activated — a mechanism that is quite distinct from that of the ribosome. A knock-on effect is that this AMM is a single-shot peptide synthesizer that cannot simply be supplied with amino acid building blocks and used repetitively; it is worth noting, however, that fuel consumption is not a prerequisite for an AMM that performs a synthetic task. One could imagine different classes of synthesis-performing AMMs: self-contained devices that are primed to synthesize a single molecule,

but lie dormant until they are activated by a stimulus, such as a change in the pH of their environment; or machines that process energy, perhaps in the form of energy-rich small molecules (such as ATP for example) or by absorbing photons of light, and can perform repetitive synthesis to create products that may lie energetically uphill from their starting materials.

Whereas ribosomal translation builds peptides from the N- to the C-terminus, Leigh's system runs in the opposite direction. This seemingly minor difference has important implications — namely, that AMMs are not obliged to imitate the mode of polymer synthesis found in living organisms; indeed, they are not even limited to nature's covalent bond-forming reactions and the production of biopolymers. It is tempting to consider the prospect that future generations of AMMs may be able to tailor-make oligomers or polymers with well defined and information-rich primary structures that have no precedent in biology. Harnessing synthetic machines could, in principle, enable access to new materials that are as complex as those found in nature, but are constructed of entirely different building blocks, and perform tasks that are only limited by the imagination and ingenuity of the chemist.

Nature exerts exquisite control over the sequences (primary structure) of biopolymers, and exploits to great effect their distinctive architectures (secondary structure), which result from folding, dictated in part by their primary structures. The sequences of many man-made synthetic polymers (polystyrenes, for example) are featureless by comparison (Fig. 1c,d). The majority of synthetic polymers, encountered in everyday life as plastic materials, are made of a single monomer and do not fold in any specific way. Although it is possible to design polymer strands that self-assemble into aggregates (tertiary structures) of well defined shape, such as spheres or cylinders[10], it is currently beyond our reach to create wholly synthetic polymer strands with secondary structures[11] anywhere close to the intricacy of a protein's. The ability to make information-rich polymers would take us a step towards this lofty goal; it is unclear, however, whether wholly synthetic systems could ever reach a level of sophistication comparable to that present in nature.

In the shorter term, time-resolved spectroscopy could be used to study the operation of these first-generation peptide-synthesizing AMMs, to determine more precisely how stochastic motion influences their operation, which may have implications for the understanding of biological motor-molecules[12–14]. Although designing systems that (i) exploit alternative covalent bond-forming reactions, (ii) create non-natural oligomers and (iii) are capable of repetitive operation will be challenging, it could open up an unexplored realm of chemistry. ❐

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