MOLECULAR MACHINES

Molecules bearing robotic arms

Mass production at the nanoscale requires molecular machines that can control, with high fidelity, the spatial orientation of other reactive species. The demonstration of a synthetic system in which a molecular robotic arm can be used to manipulate the position of a chemical cargo is a significant step towards achieving this goal.

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The development of the assembly line spurred an industrial revolution that transformed society. Richard Feynman[1] suggested that such constructs on the nanoscale could also transform how science is conducted and eventually lead to a technological revolution. Although biological systems have been using assembly lines for eons, and some synthetic DNA robotic systems have been reported[2], the progress in small-molecule-based mass-production assemblies has trailed significantly behind. This can be attributed to the 'sticky fingers' and 'fat fingers' problems[3] — not only do molecules and atoms have a tendency to stick to one another, limiting precise positional control, but also the space surrounding an individual molecule is quite small, so there is limited room for other manipulating molecules to get to work — as well as the difficulty in designing and implementing molecular architectures that can mechanically control the position and/or reactivity of other chemical fragments.

Now, writing in *Nature Chemistry*, David Leigh and co-workers[4] have taken some steps towards the challenge of creating a small-molecule assembly line by demonstrating how a molecular machine containing a robotic arm can be used to move a chemical cargo, in this case 3-mercaptopropanehydrazide, from one docking station to another. By using two orthogonal and reversible reactions — cleavage and formation of a disulfide bond and acid-catalysed exchange of a hydrazone group — the cargo can be controllably loaded and unloaded at the docking sites. When combined with the movement of the robotic arm, the cargo can be displaced 2 nm from its initial position without it ever detaching from the machine, which means that scrambling of cargo between different machines cannot happen. This last requirement is imperative for ensuring the fidelity of the transport, in that the movement of the cargo can only arise from the mechanical motion of the robotic arm.

An essential requirement for the operation of this molecular machine is that the robotic arm can move in a controllable fashion between defined positions. To achieve such function, Leigh and co-workers built their system around a hydrazone-based switch developed[5] by the Aprahamian group. A crucial aspect of these molecular switches is that a stator and rotor can be held in fixed relative orientations in their activated states through intramolecular hydrogen bonds (Fig. 1). The unsung hero in this current work — and indeed any switch/machine-related paper — is the synthetic process by which the compound is made. Without going into much detail, Leigh and colleagues mention that the flexibility, rigidity and non-planarity of the components incorporated into the machine were necessary for it to work properly — a conclusion that was likely reached only after many synthetic iterations.

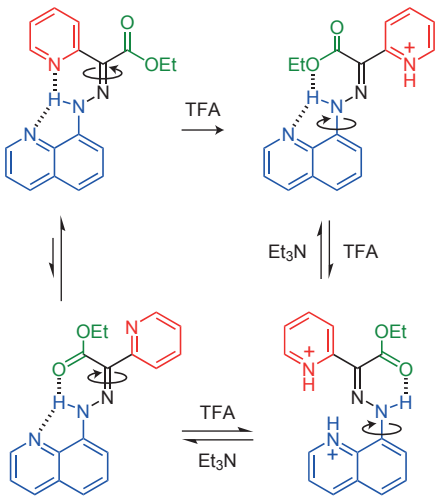

Figure 1 | A hydrazone-based molecular switch. The intramolecular hydrogen bonds position the pyridyl ring in the rotor and quinoinyl ring in the stator on the same side (top left; the *E* isomer is the major species in solution) at the onset of the switching process. Protonating the pyridyl ring results in an isomerization around the C=N double bond (top right). Excess acid leads to the protonation of the quinolinyl ring and a rotation around the C–N single bond bringing it close again to the pyridinium ring (bottom right). Excess base deprotonates both heterocycles and yields the metastable *Z* isomer (bottom left) that equilibrates with time to give the *E* isomer. Throughout the process, the quinolinyl ring is not free to rotate but locked in place in one conformation or the other through hydrogen bonding. TFA, trifluoroacetic acid.

The movement of the robotic arm (Fig. 2) involves a multistep switching cycle that changes the configuration (that is, rotation around a C=N double bond) and conformation (that is, rotation around a C–N single bond) of the hydrazone switch. Starting from compound **1**, in which the major species in solution has the robotic arm on the opposite side to the cargo, protonation of the pyridyl ring of the robotic arm (the rotor) switches the configuration of the C=N bond and leads to a single species (**2**) in which the two thiol groups are in close proximity on the same side of the stator (the platform containing a quinolinyl ring and two different aldehyde docking stations). Adding I_2 (an oxidant) to the mixture allows the mechanical arm to grab the cargo through the formation of a disulfide bond and also locks the system through the formation of a macrocycle (**3**). Addition of a large excess of acid dislodges the cargo from the docking station through the hydrolysis of the C=N bond, leaving an unbound aldehyde on the platform and a hydrazide-functionalized payload attached to the robotic arm.

Concurrently, the robotic arm rotates 180° (conformational change) as a result of protonation of the quinolinyl ring in the platform, bringing the cargo into close proximity with the second docking station (**4**). The formation of a C=N bond through coupling of the hydrazide on the robotic arm and the aldehyde on the platform locks the system in place through the formation of another macrocycle (**5**). At this stage, base (Et_3N) is used to neutralize the system and then tris(2-carboxyethyl)phosphine (a reducing agent) is added to break the

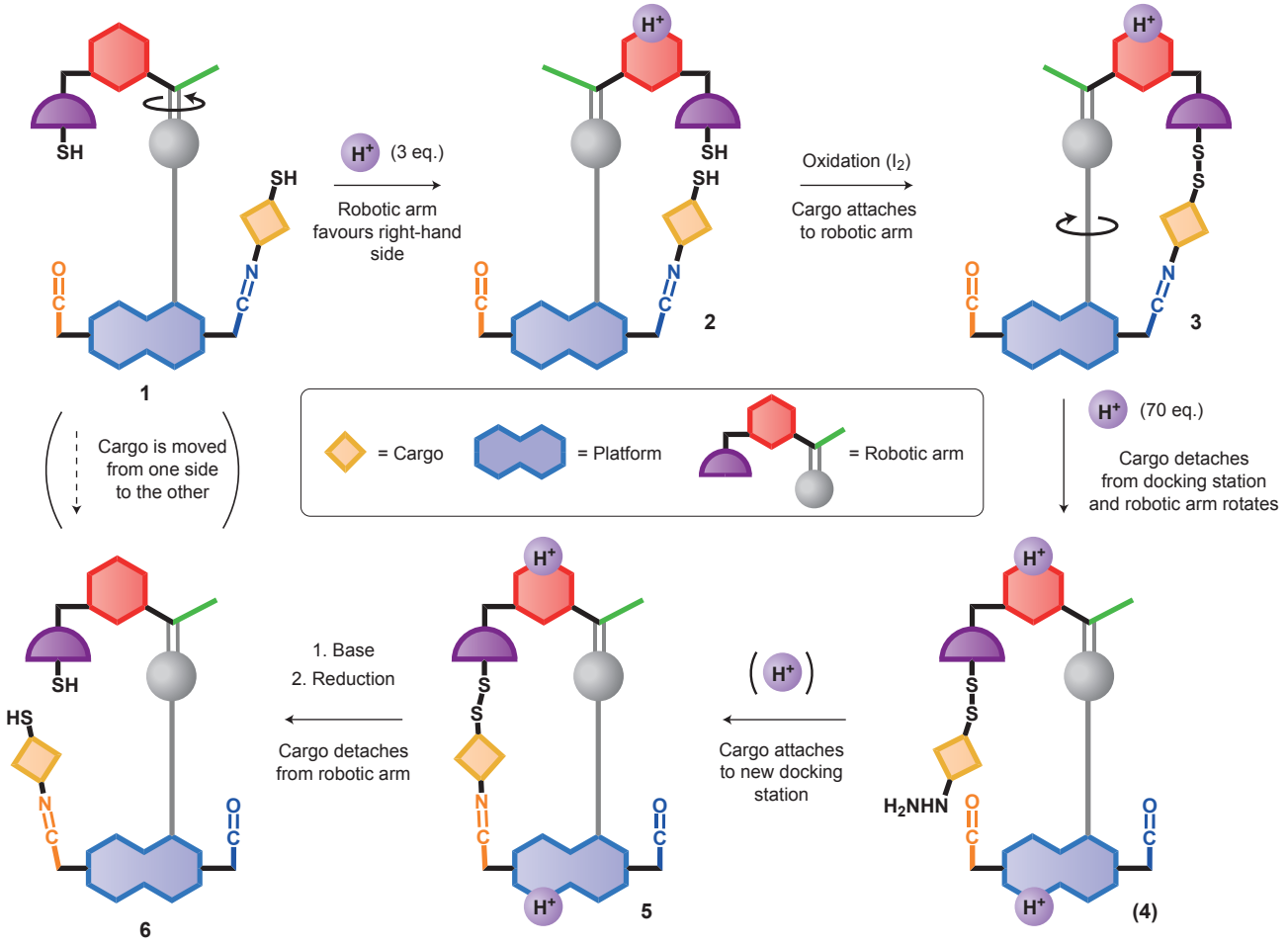

Figure 2 | A multistep switching sequence enables a robotic arm to shift a cargo from one aldehyde docking station to another. Acid-promoted isomerization around a C=N double bond (to give **2**), followed by oxidation yields the disulfide-containing macrocycle (**3**). Further addition of a large excess of acid dislodges the cargo from its docking station (**4**, not isolated) and, following rotation around a C–N single bond, the cargo anchors itself to a new docking station to form macrocycle **5**. Neutralization and subsequent disulfide reduction detaches the cargo from the robotic arm to give **6**, which differs from **1** only in the position of the cargo, that is, it is now attached to the orange aldehyde rather than the blue aldehyde.

disulfide bond, which releases the cargo at the second docking station, thus finalizing its transport to give compound **6**. This whole process can be reversed by following a sequence of oxidation (reforming the disulfide bond), protonation (configurational and conformation change accompanied by hydrazone hydrolysis and reformation) and reduction (cleavage of the disulfide bond). It should be noted that the reverse process is much slower because the acid-catalysed hydrazone exchange takes six days to equilibrate in the less acidic conditions required for switching in this direction. Nonetheless, the multistep switching cycle is very efficient and results in 79–85% of the cargo being transported (in either direction) between the two docking stations on the platform.

The cargo transport can be done in a stepwise manner — which enables the isolation and NMR spectroscopic characterization of the product of each step — or in one pot by the sequential addition of appropriate reagents. By using mass spectrometry, it is shown that at no point during transport does the cargo detach from the machine or exchange with the environment, thereby confirming the proposed robotic-arm mechanism.

One robotic arm does not constitute an assembly line, but, as has been pointed out by Leigh and co-workers, if a cohort of these machines could be lined up along a track then it may allow for the delivery of cargo (for example, substrates for catalysis) over longer distances. There are, however, many challenges — and hence opportunities — that need to be addressed before such functional synthetic assembly lines become a reality. For example, advances are required in designing synthetic molecular clocks, feedback loops, waste management and molecular compartmentalization protocols, in addition to molecular level pH gradient manipulation (among other challenges). Expanding the types of orthogonal (and/or dynamic) reactions that can be used to control the function of molecular switches and machines will also broaden the horizon of possible applications.

A better understanding of how to carefully design and manipulate energy ratchet systems[6] to enable unidirectional motion that is not limited by equilibrium isomer ratios will also be imperative. Lastly, moving away from the confinement of a reaction flask to surface-bound systems or microfluidic devices would almost certainly increase the practical utility of such molecular machines. These are exciting times for the field of molecular switches and machines — we are witnessing more and more a departure from the historic activation of single components in solution to the dynamic

and synergistic control of a multitude of processes in concert[7]. This systems chemistry[8] approach is destined to push the boundaries of what synthetic molecular machines can and will accomplish. ❐

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