Biologically inspired molecular assembly lines

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This paper proposes a biologically inspired 'molecular assembly line' — an externally programmable polymeric chain along which molecules are shuttled between chain sites along an arbitrary pathway. Our hope is to construct a scaffold that mimics biological enzymes known as polyketide synthases in their ability to physically hand-off molecules between assembly sites or domains, thereby making all chemical reactions domain-specific and diffusionless processes. It will be shown that a system with three wavelength selective chemical interactions allows a 'shuttle' on the assembly line to move bi-directionally and be bi-stable in the absence of inputs. Potential applications of the molecular assembly line in programmed assembly and molecular electronics will be discussed, as well as initial experimental work towards building a molecular assembly line driven by UV-light.

1. Introduction — biological inspiration

For years now, researchers have been pushing the physical size and complexity limits of what they can engineer. Many envision molecular-scale nano-machines — billionths of metres in size — that function as analogues to macro-scale systems, such as assembly lines that are capable of picking and placing individual molecules at will. Even though a nano-world may seem dreamlike, it is not. One only needs to look at the amazing biological world, which performs complex mechanical functions and logical operations at this size-scale at astounding speeds and with near-perfect fidelity. Thus, as we continue to push the envelope, it is important to learn from biological assembly processes or even utilise biological molecules to assemble structures.

Biology is full of enzymes that can be considered the world's smallest and most complex assembly lines. The molecular assembly line proposed in this paper is an attempt to construct a synthetic analogue to one of Nature's assembly lines, the polyketide synthase (PKS). What is a polyketide synthase, and what makes it so special? A PKS is an enzyme that produces very large and complex antibiotics known as polyketides (PKs), many of which are commercial pharmaceutical products today. Figure 1 shows an assembly line model of a PKS, where the molecule 6-deoxyerythronolide (6-DEB) is passed between domains of the enzyme as it is synthesised in a linear fashion and then cyclised. This 6-DEB molecule is a precursor that can later be tailored into many different antibiotics, in this case, the common antibiotic erythromycin [1]. If we do a back-of-the-envelope calculation using literature-reported PK production rates [2], we will find that the enzyme produces approximately one perfect drug every ten minutes from start to finish. This is an astounding rate, in the order of 10^4 times faster than commercial drug synthesis of a molecule of similar size and complexity.

How does a PKS synthesise such antibiotics with such speed and accuracy? Firstly, every reaction is catalysed, thereby speeding up the reaction. For a chemical reaction to occur, an activation energy barrier must first be overcome. Catalysis lowers the activation energy required for a reaction to occur without changing the beginning and end states of the reaction. This ultimately has the effect of speeding up the reaction rate for an ensemble of molecules.

> it is important to learn from biological processes

Secondly, the molecule is physically positioned in the pockets of the enzyme such that only stereo-selective reactions occur. Stereo-specificity refers to the 3-D nature of molecules. If we rearrange the four vertices of a tetrahedron (label them A-D, for instance), the 3-D (stereo) orientation of each vertex with respect to one another can change such that the two tetrahedrons cannot be superimposed in space. Likewise, if we take an atom that is bound to four others and rearrange the four vertices, the 3-D orientation can be changed, and this drastically changes the interactions between mole-cules in biology. For pharmaceuticals, stereo-selectivity is a measure of complexity; a drug whose bonds are not oriented properly may be ineffective, or even lethal.

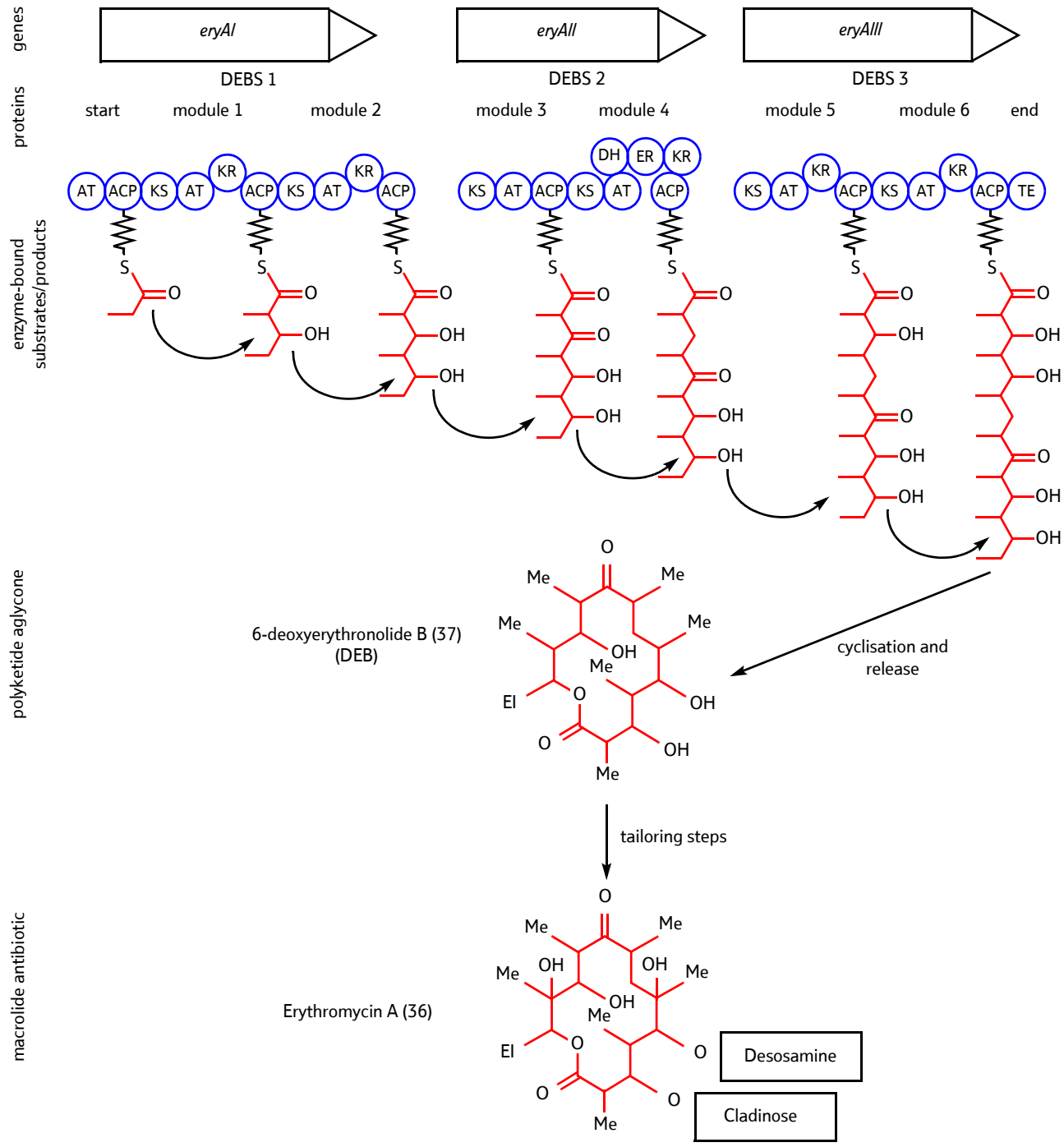

Fig 1 Assembly-line model of a polyketide synthase (PKS). 6-deoxyerythronolide (6-DEB) is synthesised linearly, and then cyclised. The 6-DEB precursor can be tailored into several antibiotics, in this case erythromycin A. Figure taken from Hopwood [1].

Thirdly, the assembled molecule is handed off between modules, thereby eliminating diffusion in the system and making the reaction domain-specific. The molecule is physically constrained such that the only reaction that occurs is the one for which the module is designed.

Furthermore, the molecule is handed off to the (n) module only once the reaction in the (n–1) module is complete, making the reaction self-clocking.

While we are interested in systems that are auto-catalytic, stereo-selective, domain-specific, diffusion-less, and self-clocking, we have chosen to initially design a diffusionless and domain-specific system. The proposed synthetic molecular assembly line is a scaffold that mimics the PKS in its ability to physically hand off a molecule or object being assembled.

This paper describes the design requirements, potential applications in molecular electronics and programmed assembly, and initial experimental results towards our goal.

2. Molecular assembly line design and potential applications

2.1 General design

Since there is no 'molecular assembly line' to our knowledge in the literature, here we are arbitrarily defining it as a system capable of moving a molecular 'shuttle' (e.g. an individual molecule or nanoparticle):

- from one monomer position along the polymer chain (monomers, by definiton, are subunits of polymers) to any other monomer position for the purpose of assembling chemical species (e.g. organic molecules, biomolecules, nanoparticles) on to the shuttle,
- along an arbitrary pathway determined by a programmed set of external inputs (i.e. the movement must be bi-directional and reversible).

Furthermore, the system should ideally be soluble in some solvent in order to maximise the total yield (i.e. not confined to a 2-D surface). The general scheme for the molecular assembly line is shown in Fig 2. The circle (with the *S*) represents the object being moved, the shuttle. The scaffold is a polymer chain composed of a repeating pattern of 3 concatenated monomer units or 'antennae', shown in parentheses. In the absence of any input, the shuttle has an equal binding affinity to any antenna, and its most stable position is tethered between two adjacent antennae. Each input frequency (ν) or wavelength ($\lambda = 1/\nu$) selectively 'turns off' binding interactions between the antennae and shuttle (i.e. $h\nu_1$ turns off the bond between the shuttle and antenna 1, where h = Planck's constant = 6.63E-34 joules * second, and $h\nu_1$ = resonance energy of the bond).

Let us say that the shuttle is in position *a*, located between antennae 1 and 2 of the first (1-2-3) repeated pattern. Upon irradiating the system with input $h\nu_1$, the bond between antenna 1 and the shuttle (*S*1) is broken, but the shuttle is still anchored to antenna 2. If the shuttle reforms its bond with antenna 1, it is once again broken. However, the shuttle is in a stable position each time it toggles to position *b*, tethered between antennae 2 and 3. Therefore, input $h\nu_1$ biases the shuttle to move to the right. We call this a 'tethered walk'.

It can be seen in Fig 2 that the system is bi-directional. The shuttle, now in position *b*, can be moved to position *c* or back to position *a* by irradiating the system with $h\nu_2$ or $h\nu_3$, respectively. Thus, if the initial position of the shuttle is known, it can be moved to any other position along the polymer chain by an arbitrary pathway as defined by the input sequence. As long as the system has three resolvable inputs that can turn off monomer/shuttle interactions, the molecular assembly line is completely externally programmable. Note that bi-directionality and reversibility are requirements for movement along arbitrary pathways. If the shuttle can only

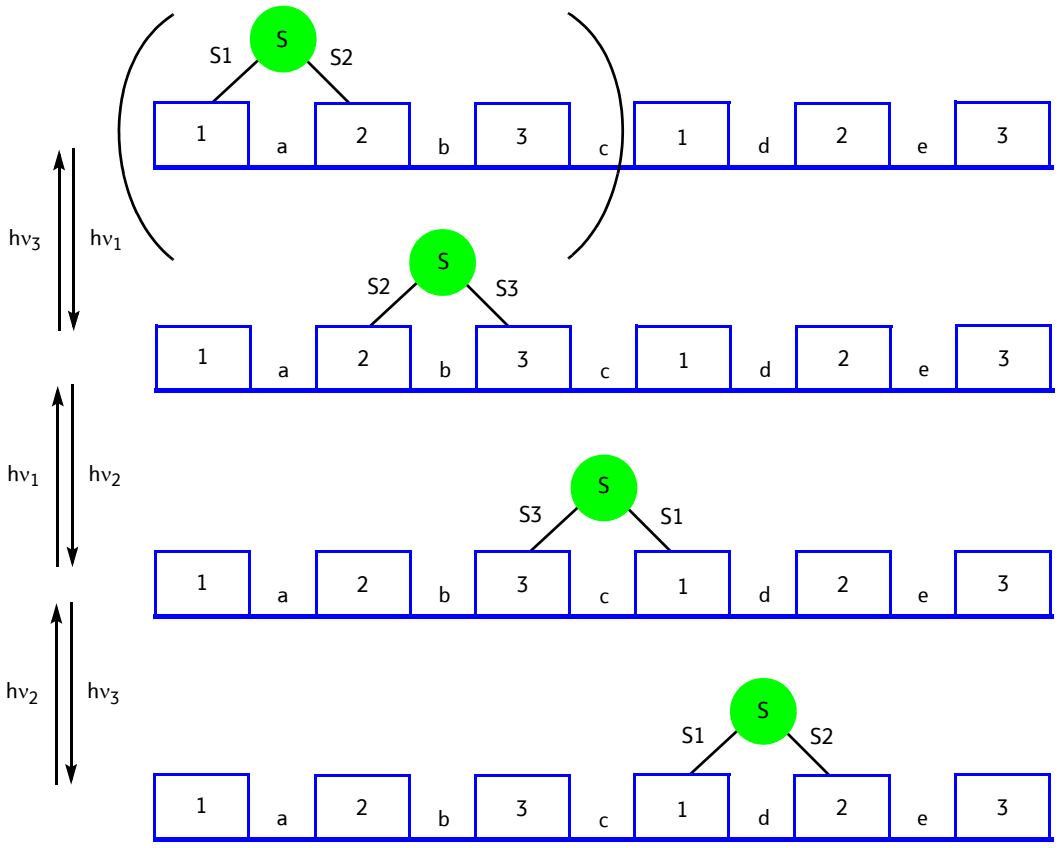

Fig 2 General scheme for a molecular assembly line. The circle represents a shuttle (*S*), and each numbered rectangle represents a different antenna. *S*1, *S*2, and *S*3 represent the bonds formed between the shuttle and each of the antennae. Lower case letters represent stable positions where chemical reagents can react with the shuttle. The repeating pattern is shown in parentheses.

move to the right of its current position, locations to the left cannot be reached unless the linear chain is circularised. Even if the chain is circularised, the shuttle cannot move along arbitrary user-defined pathways in a uni-directional and irreversible system.

2.2 Potential applications

It has already been demonstrated that physically constraining reactive molecules greatly increases the reaction efficiency [3—5] in synthetic systems. Liu et al have performed DNA-templated syntheses where DNA-bound reagents react when two complementary DNA single-strands form hybridised double-strands, and have successfully programmed multi-step chemical reactions [3] with stereo-selectivity [4]. They have also performed selective 'one-pot' syntheses, in which several very specific reactions occurred simultaneously in one solution even though the individual reactants would be reactive with one another without DNA-templating [5].

The molecular assembly line is a scaffold that physically localises a chemical functionality such that the reactions are domain-specific. Our system was originally designed for programmed chemical assembly schemes as in Fig 3, where the set of inputs programs the sequence of a DNA molecule synthesised on a bead. In Fig 3, individual nucleotides are located along the backbone between antennae (lettered positions in Fig 2). Firstly, the shuttle positioned at *a*, and then the polymerase enzyme (under certain conditions), adds the nucleotide to the growing chain. The user then decides to add the nucleotide at position *d*. The input sequence $h\nu_1$-$h\nu_2$-$h\nu_3$ is pulsed in order to move the shuttle to position *d*, and then the enzyme once again adds the nucleotide to the growing chain.

> we envision a system that relies only on wavelength selectivity

One could imagine positioning reagents using tools such as 'optical tweezers' that could effectively trap one bead with one laser [6]. However, due to its limited scalability, this technique is not well suited for our purposes because we would like to control at least billions of reactions simultaneously. We rather envision a system that relies only on wavelength selectivity — only one global input addresses an entire solution volume without any spatial resolution of the input required.

The molecular assembly line can also be used in molecular electronics. The simplest (1-2-3) pattern is a bi-stable toggle switch. If one could load a shuttle on to the first (1-2-3) group, the assembly line could be used as a molecular shift register where a shuttle represents a physical bit, where the absence/presence of a shuttle at a particular position represents a zero/one at that register; all bits would be shifted in parallel by consecutively pulsing $h\nu_1$-$h\nu_2$-$h\nu_3$. It should be noted that this non-volatile molecular shift register memory greatly resembles the volatile electronic shift register memory originally proposed by Hopfield at Bell Laboratories [7].

3. DNA 'walking' devices

Schemes similar to the molecular assembly line have recently been reported in the literature [8—10]. These devices are DNA machines in which a DNA 'walker' moves along another DNA 'track'. Reif et al have reported autonomous, uni-directional [8] and bi-directional [9] walking devices that operate without any external inputs; Seeman et al have reported a programmable, bi-directional DNA 'biped' that uses a third DNA 'fuel' molecule to direct the movement of the walker DNA molecule along the DNA molecule track [10].

Our proposed design varies slightly from those in recent literature reports. The systems reported by Reif et al are autonomous and require no input, whereas our proposed system is externally programmable; the shuttle can move along arbitrary pathways, and can furthermore be started/stopped at will due to its bi-stability.

The system reported by Seeman et al is externally programmable using a chemical input of DNA 'fuel' strands in solution, as opposed to our molecular assembly line, which is electronically controlled via irradiation. However, we find these recently reported results extremely exciting, and it should furthermore be stated that none of the designs are mutually exclusive.

4. Towards building a molecular assembly line

The two things required to build a molecular assembly line are:

- monomers that can be polymerised into the (1-2-3) group, which is then further polymerised into the assembly line backbone,
- wavelength- or frequency-selective control of monomer/shuttle interactions.

Our initial experimental efforts have been directed towards developing a set of monomer antennae whose interactions with a shuttle can be controlled by the wavelength of ultraviolet light.

Selective control over monomer/shuttle interaction is conceptually shown in Fig 4a. As in Fig 2, *S* is the shuttle. 1, 2, and 3 are the three antennae. *S*1, *S*2, and *S*3 represent the complexes formed between the shuttle and each respective antenna, where the bonds/interactions between the shuttle and each antenna are broken when irradiated with light of the appropriate wavelength (or frequency). If a solution of *S*1 is irradiated with input 1, the complex is broken up into the two compounds, *S* and 1; when the light is turned off, *S* and 1 reform to give back *S*1. Because these reactions are competitive, light can control the relative equilibrium between the *S*1 formed by the reaction of *S* and 1, and the individual amounts of *S* and 1 formed by the photocleavage of *S*1 by input 1. The same can be said for systems involving *S*2 and *S*3.

Figure 4b shows the shuttle and set of monomer antennae that we have designed to prove the concept outlined above, as well as the respective wavelengths to break, or photocleave (cleave by light) the bonds between the shuttle and each antenna. The bonds between the shuttle and antennae are formed and broken based on the special photochemistry of carbonyl compounds. The molecules *S*1 (alkyl ketone), *S*2 (aryl

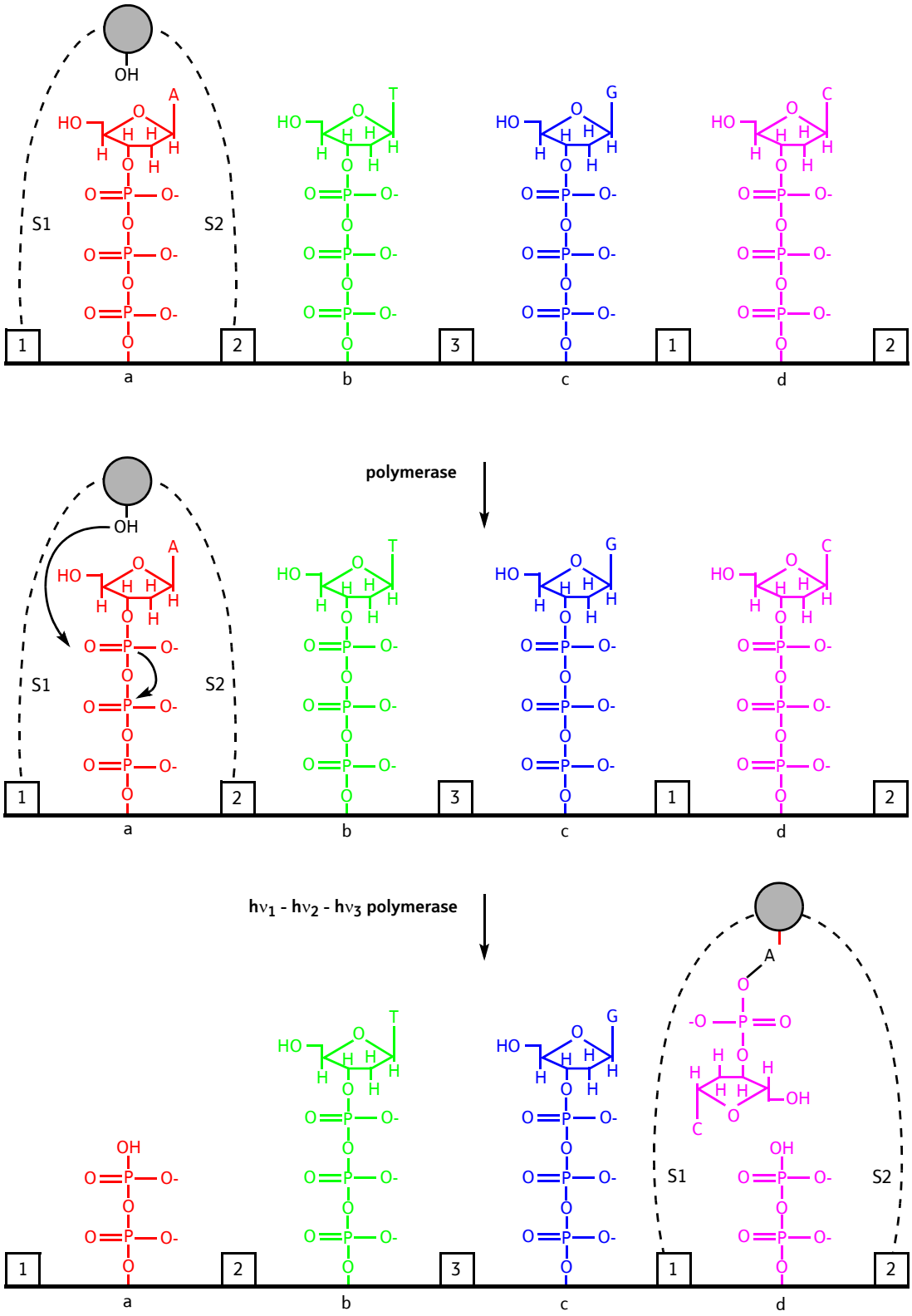

Fig 3 Idealised DNA assembly scheme using the proposed molecular assembly line. *Top* — the shuttle is positioned over the nucleotide in position *a*. *Middle* — polymerase is added and the nucleotide is now on the shuttle (a small diphosphate molecule is left on the backbone). *Bottom* — the assembly line is programmed to add the nucleotide at position *d*. The support is properly positioned firstly by pulsing inputs 1, 2 and 3 in succession, and then the addition occurring again.

ketone), and *S*3 (amide) are all carbonyl compounds that have been designed to photocleave at wavelengths of approximately 280 nm, 310 nm, and 210 nm, respectively. In the presence of a catalyst (a lithium salt), the shuttle and each antenna can reform their bonds by the so-called Michael reaction.

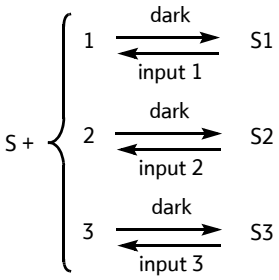

Fig 4a Conceptual scheme for the competitive addition and photocleavage reactions. The shuttle and each antenna can form bonds, but these bonds can be broken by light.

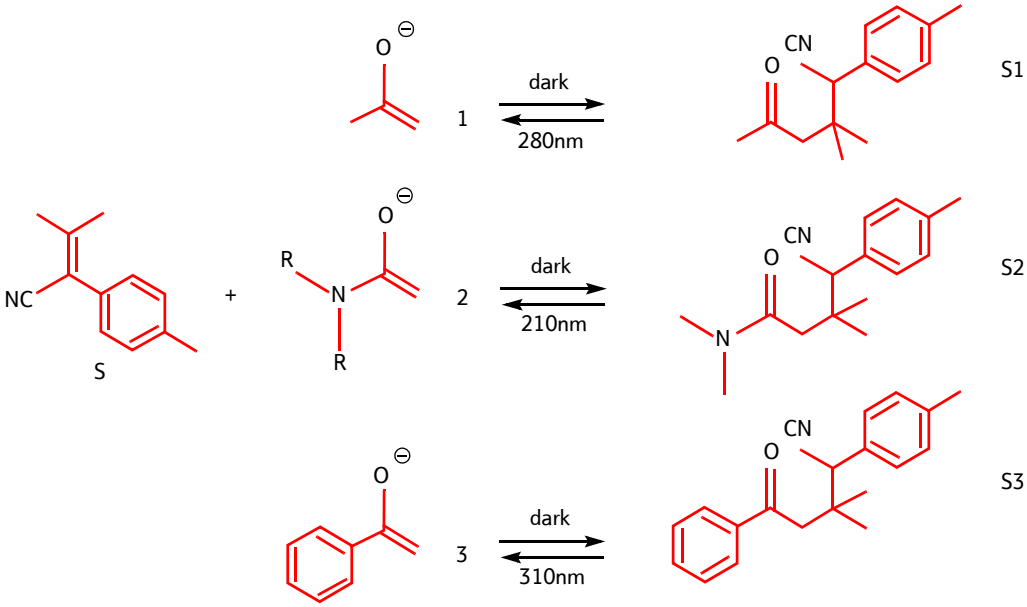

Fig 4b The custom-designed carbonyl compound used in the experiment.

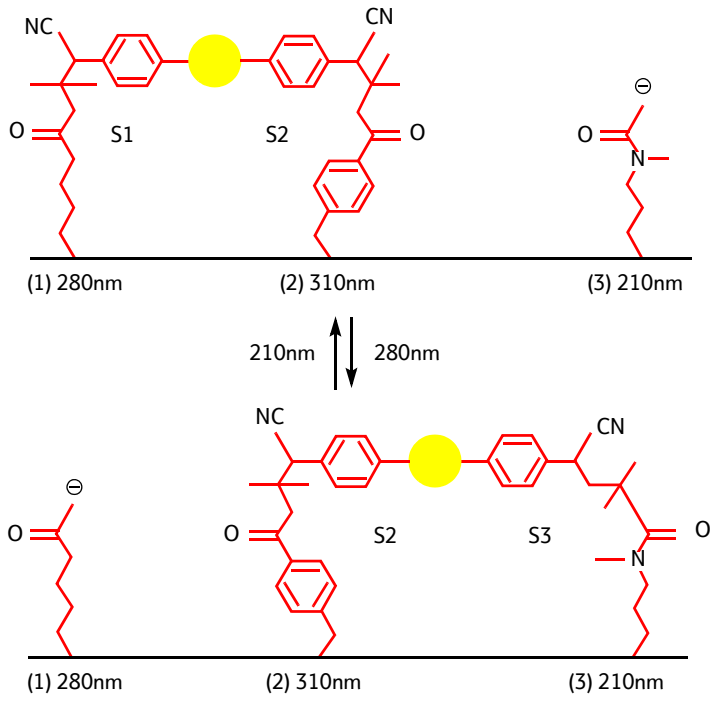

Fig 4c The repeating (1-2-3) unit of a molecular assembly line based on the wavelength selective photocleavage of the carbonyl compounds in Fig 4b.

Figure 4c shows one idealised (1-2-3) repeated sequence of a molecular assembly line employing these designed molecules.

In our initial proof-of-concept experiments [11], a mixture of *S*, 1, and *S*1 were prepared. A catalyst (lithium salt) was added to facilitate the reaction between *S* and 1 to reform *S*1. One mixture was reacted in the dark, while another was irradiated with λ_1 UV-light. The relative amounts of *S* (the amount of *S* and 1 are equal) and *S*1 were determined by high-performance liquid chromatography (HPLC). HPLC is a technique that separates a mixture, and then produces a plot in which peak area corresponds to the relative amount of a compound in the mixture.

One would expect the dark reaction mixture to have a higher ratio of *S*1:*S* than the reaction that proceeded in the presence of UV-light since the input controls the relative equilibrium amounts. The reaction between *S* and 1 forming *S*1 is in competition with the photocleavage reaction that breaks *S*1 into *S* and 1.

Figure 5a shows HPLC chromatographs of the reactions for *S*1 in the presence or absence of UV-irradiation. As expected, the dark reaction has the higher *S*1:*S* ratio, which can be seen by the relative peak sizes (corresponding to amount) of *S*1 (right peak) and *S* (left peak). Thus, we have demonstrated the ability to control the equilibrium between *S*, 1, and *S*1 by UV-irradiation.

The same is true for compound *S*3 (Fig 5b). Furthermore, *S*1 does not cleave in the presence of input 3, nor does *S*3 cleave in the presence of input 1. Since the binding and cleavage mechanisms for *S*2 are also the same, we are confident that a chemical system with three resolvable inputs is possible.

There are many future challenges before the dream of a molecular assembly line can be fully realised. Here, we have demonstrated control over monomer/shuttle interactions with wavelength selectivity for one particular group of small molecules, but this control must be fine-tuned.

Furthermore, other types of monomer antennae and shuttles may eventually prove better candidates than the ones presented here. For example, we have shown that it is also possible to selectively dehybridise DNA (separate a DNA duplex) by attaching a 1.4 nm gold nanoparticle antenna to a DNA molecule and putting it in a 1 GHz RF magnetic field [12]; the RF inductively couples to the nanoparticle, and heat is transferred from the particle to the DNA, thereby dehybridising the duplex.

A summary of many potential candidates can be found in Chow [11]. Also, a synthetic strategy to polymerise the monomer antennae into the necessary backbone must be developed. However, Weck et al have recently developed a particularly promising synthetic strategy towards such a polymeric scaffold [13]. Another possible strategy is to build the scaffold out of DNA by constructing monomer units that resemble the DNA complexes of Liu et al [3—5].

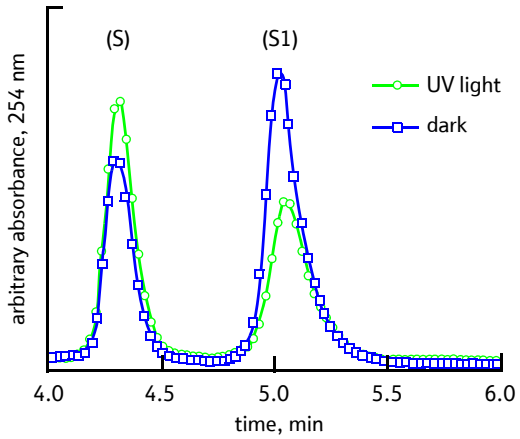

Fig 5a HPLC chromatographs monitoring the equilibrium between compounds *S*1 (right peak) and *S* (left peak). The relative concentrations correlate to the peak sizes. The dark reaction has a high *S*1:*S* ratio because *S* and 1 have reacted to reform *S*1. The ratio in the presence of UV-light is lower because the reaction between *S*1:*S* to form *S*1 is in competition with the photocleavage of *S*1 into *S* and 1.

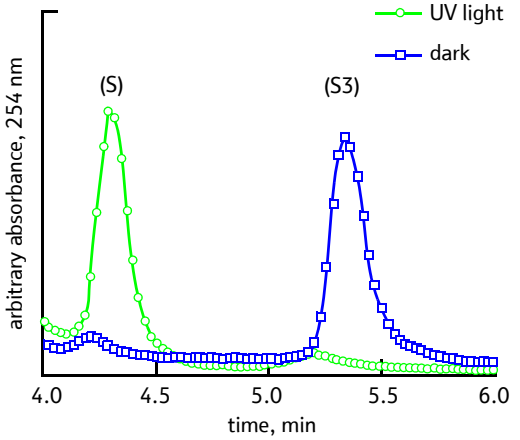

Fig 5b HPLC chromatographs for the S3 system. The same trend as in Fig 5a is observed.

5. Conclusions

We have proposed the design of a molecular assembly line — an externally and electronically programmable polymeric scaffold that precisely positions an object along the scaffold. It is an attempt to mimic the ability of an enzyme, the polyketide synthase, to physically hand off the object between modules of the scaffold such that the reactions are domain-specific and diffusionless. We have demonstrated wavelength-selective control of the relative equilibrium of a chemical reaction — the first step towards building a molecular assembly line.

We are hopeful that, in time, precise placement of individual molecules will be achievable in a scalable manner because the system can be externally and electronically programmed.

In addition to applications in programmed assembly, we envision that this proposed scheme will have many applications in molecular electronics — an interesting possibility given that many researchers are looking towards the next generation of computing as we approach the limits of

Moore's Law. To that end, we are working towards synthesising intramolecular toggle switches that could perhaps be used for laser-rewritable memory applications.

Acknowledgements

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Joseph Jacobson leads the Molecular Machines group of the Center for Bits and Atoms. His research is aimed at reinventing microelectronics by developing processes for directly and continuously printing communication, computation, and displays on to arbitrary substrates. He was educated at MIT and Stanford, receiving a PhD in Physics and a postdoctoral fellowship in Physics, respectively. As a graduate student researching femtosecond lasers, he set the record for the shortest pulse ever generated by a laser (in optical cycles). His postdoctoral work in nonlinear-nonlocal quantum systems was published in the Physical Review. He is the author of 45 peer-reviewed journal and conference papers and holds several patents and patents pending in display technology and printed electronics. He received a 2001 Discover magazine award for technological innovation, and in 1999 he was named as one of Technology Review's 100 most influential innovators under the age of 35.