Exploring the Nano World: Engineering & Design with Biology

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Introduction

This paper outlines the capacity of the scientific domain of engineering biology to produce biologically based devices and to become a new manufacturing base. This will be illustrated by discussing the design of a bio-nanostepping motor and a discussion as to how this device can be produced, evaluated and mass-produced. This paper also presents the ideas and concepts on which this is based.

We are in what is being referred to as the 4th industrial revolution, with one of the major emerging platforms being the use of biological materials and applying the principles and processes to develop a new sustainable manufacturing base. This is possible due to recent major scientific and engineering developments (Byrne et al. 2018).

Living organisms harness biomolecular self-organisation to construct and manipulate often intricate machinery and super-structures at the nanoscale. Being able to have controlled manipulation of the shape, size and interactions of such super-molecular building blocks opens up extensive potential for in vitro artificial self-assembled nanoscale material. As such, these organic units can be incorporated into a living building.

The current industrial manufacturing context is one that faces environmental, political, geographical and economic factors. Society is also changing its perception of global energy use and manufacturing practice (RAE report 2019). There is a concomitant movement towards reducing the size of components to the nanometer scale, using and manipulating individual molecules or atoms to perform specific tasks. Technological developments, particularly in using biology, are being applied to the challenges faced by human society. A specific reason for this is that biological molecules and systems processes have advantages over man-made components and devices and current manufacturing methods. The advantages include being able to operate at the atomic/molecular level, often using energy more efficiently, not requiring high temperatures or pressures to operate; in addition, the self-assembling and regulating processes are less technologically complex and less environmentally polluting (Clomburg et al. 2017, Byrne et al. 2018, Subramani and Ahmed 2018) Biological materials, in certain circumstances, also have another major advantage over non-biological materials: this is their biodegradability (UK BIA report 2018). A major challenge is to extract and then re-assemble these components into an integrated working mechanism; furthermore, any device needs to be controllable.

All machines need energy to work. The most abundant, freely available and environmentally friendly energy source is solar energy. Much of the biological world relies on photosynthesis to provide the energy they need to operate and live. The ‘light reactions of photosynthesis’ involving chlorophyll are responsible for trapping visible light energy and moving the energy within the plant with an efficiency of more than 90%. This energy could then be transformed into other types of energy, such as electrical or the chemical energy of adenosine triphosphate, ATP (Shevela et al. 2023).

In the last few years techniques have developed such that we can now detect an individual molecule. These can also obtain information on the detailed structure, properties and behaviour of these molecules. Modern super-resolution microscopic techniques enable live cells to be investigated, thus providing dynamic information on biological processes (Schermelleh et al. 2019, Khater et al. 2020). As a result, we can also manipulate biological

systems at the molecular level. This enables us to view biology as a big tool box similar to a Meccano set (UK BIA report 2018) with about 85 million types of proteins (https://biology.stackexchange.com/questions/58868/how-many-proteins-are-in-the-earths-proteome) being pieces which can be combined and assembled into a host of different configurations. New materials, devices and systems can be designed and produced that do not exist naturally. The new super-resolution microscope techniques are pivotal tools in synthetic and engineering biology (Lv et al. 2022). Within these domains, two main approaches are used. One is the top-down approach, which uses the biological systems inherent in living cells to produce macro-molecular assemblies. This approach is based on the techniques of genetic engineering, cellular and molecular biology. The alternative is the bottom-up approach, which does not use living cells to assemble complex biological systems (Figure 1). Here the innate ability of biological molecules to self-assemble is used, along with chemical synthesis and the physical manipulation of molecules (Damiati 2019).

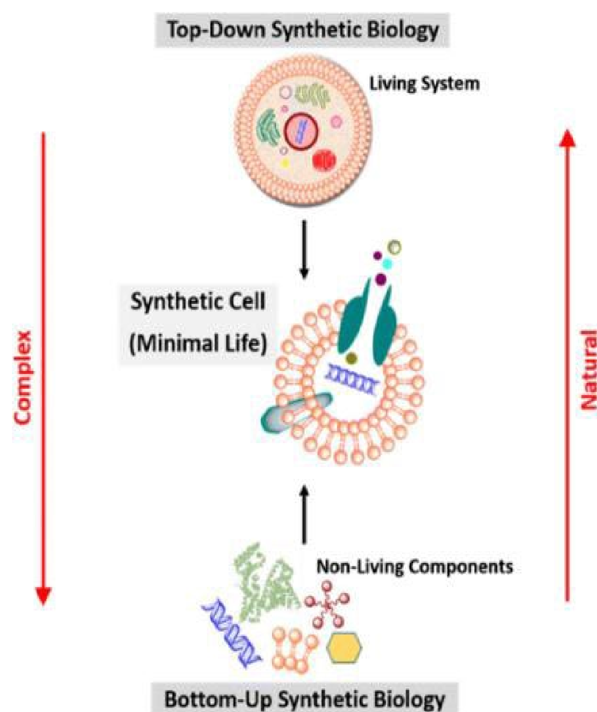

Fig 1. Damiati (2019) Diagram illustrating the two main approaches used in synthetic biology

The bionanomachine presented here is designed to move a tubule or rod in 8nm controllable steps and to operate outside of a cell. The biological principles and materials of photosynthetic light (energy) harvesting and transfer are used in creating this device. This requires the use of the ATP synthase complex for ATP production and Kinesin motor protein movement. To reverse, or reset, the rod back to its starting position, a genetically engineered kinesin, which is activated by blue light to reverse the direction of movement of the kinesin, could be used (Nakamura et al. 2014).

There are three sections to this device that can be used either in an independent (modular) concept or integrated as a functioning device (see Figure 2). The three sections are:-

A) Light harvesting and energy transfer conduit (in Figure 2 coloured green)

B) Energy conversion producing ATP. (in Figure 2 coloured blue)

C) Mechanical translation (in Figure 2 coloured red)

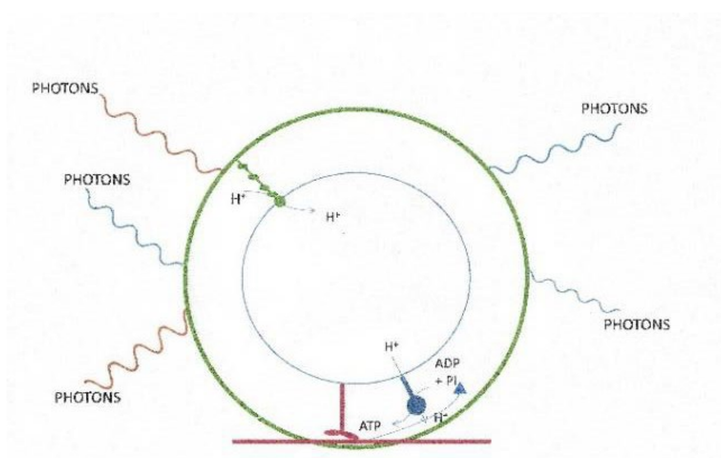

Fig 2. Sparrow (no date) Diagram showing the architecture of a bionanostepping motor. The structures in green are a membrane incorporating LHCII with an energy conduit linking the outer to an inner membrane. The components in blue are an inner membrane with an incorporated ATP synthase complex to generate ATP. The red components are a kinesin attached to a nanorod. The kinesin heads produce a step in the presence of ATP which then pushes the rod. The intermembrane space encapsulates ATP/ADP/Pi to enable ATP recycling.

One of the major challenges would be to assemble the components into an integrated working mechanism. It is proposed to employ molecular self-assembly, which is a property innate to many biological molecules. To produce such a complex macro-molecular biological architecture requires controlled, sequential, and pre-determined molecular self-assembly. It is proposed to use droplet based micro-fluidics to automate the assembly of this bionanostepping motor machine. It is also proposed to, where possible, develop integrated diagnostics and sorting systems. This will be to assay and then select components with the required technical specifications to allow each component to progress onto the next assembly stage. Then undertake a final quality assessment of the fully assemble machine.

Methodology

This section outlines the concepts on which the assembly of a biologically based nanosized machine is based, rather than specific methodologies or techniques of its construction.

Biological Molecular Self-Assembly

This is a property of many biological molecules that, by their random movement, produce complex structures. Self-assembly is where molecules spontaneously and autonomously form

non-covalently bonded stable, structured aggregates. The structures are stabilised via weak electrostatic interactions such as hydrogen bonds, hydrophobic and hydrophilic interactions and Van der Waals forces. This is often facilitated by the assembling molecules having specific complementary properties such as surface shape, functionality and charge. Self-assembly can be either static or dynamic. Static self-assembly is where, once the final stable state is achieved, no energy is required to maintain it, whereas dynamic self-assembly requires energy to maintain this state. There is also a distinction between inter- and intra-molecular self-assembly. Intra- molecular assembly is where large multi-molecular structures are produced (Karthikeyan and Waqar 2018).

Self-assembly of the light harvesting array: this would be a connective pool of chlorophyll light- harvesting complexes (LHC). Chlorophyll is a natural pigment used to absorb light and transfer the absorbed energy around a system. To accomplish this, the chlorophylls are arranged in a specific configuration, which is achieved by being attached to proteins forming an LHC. These complexes are then correctly orientated for efficient energy transfer by being incorporated into a lipid membrane called the thylakoid membrane (Shevela et al. 2023). For several years researchers have been exploring and, using self-assembly, producing artificial light harvesting systems using natural and synthetic pigments (Katterle et al. 2007, Wu et al. 2024).

Production of the energy transfer conduits: Zhou et al. (2019) demonstrated that directional long range energy transfer is possible and efficient (Figure 3). They used a double-stranded DNA- template (dsDNA) with conjugated dye aggregates of benzothiazole cyanine dye K21.

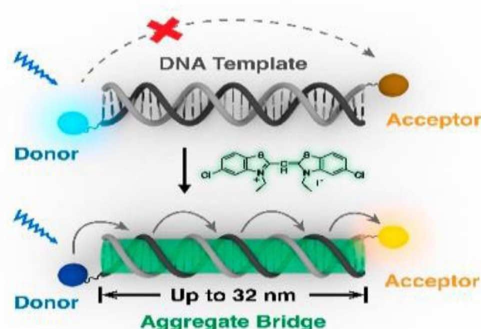

Fig 3. Zhou et al. (2019) Diagram showing an artificial light harvesting system with donor and acceptor terminal conjugated Alexa Fluor dyes along a dye aggregate bridge.

Figure 3 presents the energy transfer conduit as developed by Zhou et al. (2019). Here, this would need to be modified, it is proposed to use chlorophyll or light-harvesting complexes instead of benzothiazole cyanine dye K21. At the donor terminal end, Alexa Fluor dye could be substituted by a chlorophyll molecule. However, at the acceptor terminal end the Alexa Fluor dye would need to be substituted for a proton pump. For this, a number of different reaction centres could be used such as photosystem I (PSI), and photosystem II (PSII) from higher plants or photosynthetic bacteria, or potentially, bacteriorhodopsin could be used.

Production of double vesicles: within biological systems many functions are regulated and

managed via proteins, including enzymes. To ensure these proteins are able to correctly co-ordinate with one another they are often incorporated within a membrane. Membranes are also used to compartmentalised into functional areas called organelles. These membranes are composed primarily of lipids. Lipid molecules are hydrocarbon based and typically have two domains, one is hydrophobic (the tail) and a hydrophilic head. This configuration means that when interacting with water the hydrophobic tails will associate together away from the water molecules with the hydrophilic heads interacting and associated with the water molecules. In this way lipids will naturally self-assemble into bilayer membranes and at certain concentrations they naturally form vesicles (Watson 2015). Microfluidics are used to produce single and multivesicular vesicles where the lipid composition and contents of each vesicle can be independently controlled (Damiati 2019).

Assembly of the ATP generating system: in the mitochondria the ATP synthase complex is attached to the mitochondrial membrane. Thus, in this device, an ATP synthase introduced to a lipid membrane should self-assemble into the vesicle membrane. The core of this device is based on a modified version of the Steinberg-Yfrach et al. (1998) system of a liposome with an incorporated light activated reaction centre and an ATP synthase complex. This part of the device converts the excitation energy from light absorption into the biologically important chemical energy molecule of ATP. The proton pump mechanism (a reaction centre or bacteriorhodopsin) creates a proton motive force (a proton concentration gradient and potential difference across the membrane), which is used to activate an ATP synthase complex. This then uses the proton motive force to couple the substrates adenosine diphosphate (ADP) and an inorganic phosphate (Pi) to produce ATP. This is based on the system developed by Steinberg- Yfrach et al. in 1998. The modification for this device is that the proton pump and the ATP synthase are attached to the inner vesicle of a double vesicle system. The substrates ADP, Pi and ATP and the protons generating the PMF are enclosed within the space between the two vesicles. Thus, these substances can be recycled.

Assembly of Kinesin attachment to a vesicle and microtubule: Kinesin motor proteins are used in all eukaryotic cells to transport a variety of materials, termed cargoes, such as proteins in a vesicle. Kinesins are a superfamily of motor proteins that undergo ATP-dependent movement along a microtubule. A microtubule is a 25nm tube like structure which serve as intracellular rails on which motor proteins travel from one part of a cell to another. Microtubules are polar in that they have a fast-growing end, which is termed the plus end. The other non-growing end is referred to as the minus end. Kinesins move in a single direction from the minus to the plus end of the tubule. On hydrolysis of the ATP the motor produces a step-wise movement along the microtubule going from one binding site and then onto the next. When Kinesin moves, the head that is bound to the tubulin is not released until the other head binds to the next binding site along the tubulin. One head is always bound to the microtubule. The Kinesin motor domain binds to the tubulin at 8nm intervals. (Yildiz 2024)

Microfluidics For Controlled Molecular Self-Assembly

Microfluidic systems have channel dimensions of tens to hundreds of micrometres that enables them to manipulate nano- and pico-litre volumes of liquid. Such devices enable the physical and chemical properties of fluids to be controlled and are powerful tools for regulating the processes of self-assembly. These systems boost the efficacy of mixing, such

that efficient, proper and complete mixing of liquids is obtained, enabling the fine control of parameters such as temperature. Microfluidic devices can be used in manufacturing self-assembly molecules producing stable self-assembled structures. Microfluidics is a powerful technology for controlling the bottom-up self-assembly of nano- and micro-scale structures (Dou et al. 2017). Microfluidics offer significant advantages over conventional methods, as it provides kinetic control, allowing thousands of molecular molecules to be assembled into discrete supramolecular structures. (Khoneini et al. 2021).

Droplet microfluidics is the encapsulation of reagents encapsulated in an emulsion of discrete droplets, typically an aqueous phase in oil. With this technique, huge quantities of uniform droplets can be produced allowing high throughput analysis. The main advantage of droplet microfluidics is its highly multiplex capabilities. As reactions are confined to independent droplets, each of a few nanolitres, it is possible for thousands of reactions, or self-assembly events, to take place concurrently. As each droplet is discrete and independent, the reagents present inside a droplet can be readily tailored to fit a variety of needs. As this technique employs droplets of very small volumes, the diffusion time for a molecule is quicker than with conventional systems, leading to quicker reaction times (Convery and Gadegaard 2019). The ability to precisely manipulate droplets after their generation is key in maximising their utility as reaction, assay or storage vessels (Suea-Ngam et al. 2019). The benefits of droplet-based microfluidic reactors over continuous-flow and flask-based methods for material synthesis are well-recognised and, if made and operated in parallel over prolonged periods, would be a relatively simple way to up-scale production (Jain et al. 2002). Once droplets have been produced there are a range of manipulation techniques that can be undertaken: there are passive and active techniques, as shown in Figure 4. Droplet- based microfluidic platforms typically incorporate many functional components that allow complex experiments on a range of chemical and biological systems. This means that once formed droplets, and their internal contents, can be dynamically changed within the microfluidic device. For example, pico-injection involves injecting a specific volume of a chemical, at a pre-designated point, into a droplet. The ability to generate and analyse large numbers of droplets at high-throughput is a particular strength of droplet microfluidics (Suea-Ngam et al. 2019).

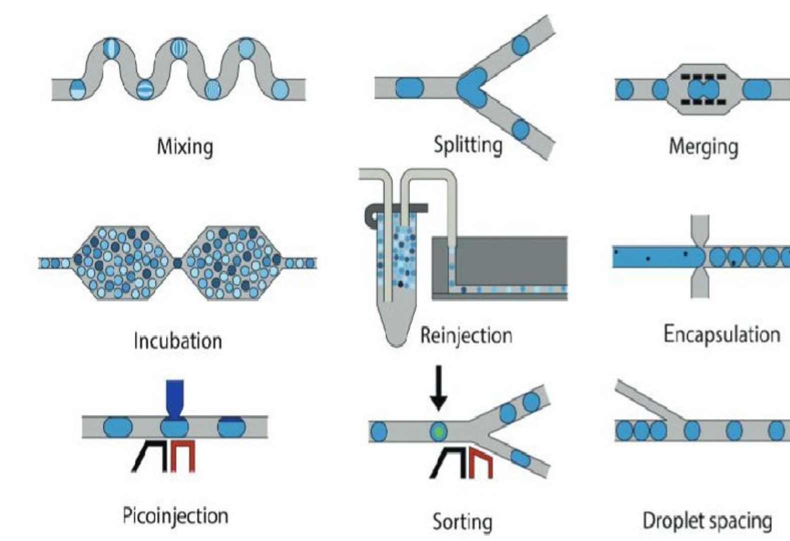

Fig 4. Suea-Ngam et al. (2019).Diagrams illustrating different channel geometries to undertake a range of droplet manipulations within a microfluidic device.

Multiple layer membrane systems have been produced using microfluidics for some time. (Matosevic and Paegel 2013)

Detection in microfluidic chips: analysis of samples is often required to monitor biological processes and, as microfluidics is being proposed as the platform technology to assemble biological components it would be useful to be able to analyse the assembly at critical stages within the same system. Incorporating optical detection methods such as absorbance and fluorescence within a microfluidic device is commonly used (Richter et al. 2023)

Diagnostics of Energy Transfer Conduit

Below is an outline of how a component such as the energy transfer conduit could be assayed as an internal diagnostic process in a microfluidic device. In this case, the assay is to ensure that energy is flowing from the donator to the acceptor terminals through the intermediary pigments.

In the system shown in figure 3, Zhou et al. (2019) reported that energy transfer was demonstrated by attaching an Alexa Fluor 350 molecule as a donor and then Alexa Fluor 555 as a terminal acceptor. Here chlorophyll molecules will be used instead of benzothiazole cyanine dye K21. Coupling fluorophore dyes to light-harvesting systems has been undertaken with Texas Red (Hancock et al. 2021). and Alexa 647 (Yoneda et al. 2015) (see figure 5). This demonstrated that energy transfer between the fluorophore and light-harvesting complexes is between 60 – 92% efficient. Here I also propose to use Alexa Fluor 350 as the donor with Alexa Fluor 750 as the terminal acceptor. Each of the different pigment components should have spectrally separate absorption and fluorescence (light emitting) characteristics such that each component is spectrally differentiated and can be specifically identified. For the donor terminal end, Alexa Fluor 350 is suitable as it absorbs light at 343nm (ultra-violet) and emits at a wavelength to excite the chlorophyll, and Alexa Fluor 750 is suitable for the acceptor terminal end as it absorbs in the wavelength region of chlorophyll fluorescence, and it has a peak fluorescence at 775nm (infra-red) (Bell et al 2015). When the dsDNA-HL conjugates are illuminated with 340nm light, and if fluorescence is observed in the 770-800nm (infra-red) region, this would show that energy has been transferred along the conduit. This is an adaptation of a widely used technique called FRET (Fluorescence Resonance Energy Transfer) to extend this energy transfer process; Zhou et al. 2019 demonstrated the principal concept of this idea was possible (see figure 3). If the Alexa Fluor 350 is not correctly coupled to the chlorophyll, then fluorescence between 400 – 500nm (blue light) will be observed. If any of the chlorophyll's are not properly connected, then fluorescence at 700nm (red light) would be observed.

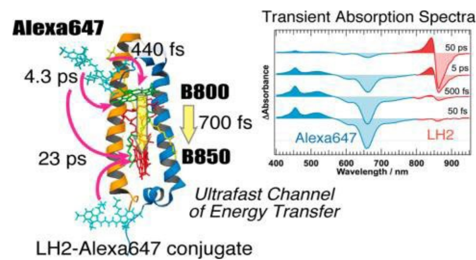

Fig 5. Yoneda et al. (2015) Diagram showing an LH2-Alexa 647 conjugate

Results

Researchers have been able to construct a number of the sub-components of the machine presented here. For example, building lipid vesicles and multi-layer vesicles using microfluidics has been possible for a number of years (Diamanti 2019). Similarly, many researchers have been experimenting with and producing artificial light harvesting arrays capable of energy transfer. These have been using natural and synthetic pigments in a variety of membrane environments (Katterle et al. 2007, Wu et al. 2024). The directed energy conduit has been designed and produced by Zhou et al. (2019). A modified version of this is proposed to be the basis for the energy conduit in the bionanostepping motor presented in this paper. ATP production outside of a cell has been shown to be possibly by Steinberg-Yfrach et al. (1998) when they attached an ATP synthase complex to a lipid vesicle with an incorporated bacteriorhodopsin as a proton pump. They were able to show light induced proton pumping to produce a proton motive force (PMF) which then generated ATP. Lu and Jewett (2023) have shown that ATP / ADP recycling within an encapsulate vesicle system in an enclosed space is possible.

Discussion

This paper outlines a potential architecture of a bionanomachine (Figure 1). Importantly, the principles on which it could operate and the processes by which it could be mass produced.

The flow of energy through the system is as follows. Light is the primary energy to drive the machine and is harvested by the photosynthetic light harvesting pigments incorporated within the outer membrane of a vesicle. The excitation energy is then transferred to the inner membrane via a specific directional energy transfer conduit. At the terminal end of the conduit is a photoactive proton pump, potentially a bacteriorhodopsin. On activation, the bacteriorhodopsin pumps protons from the space between the two membrane vesicles and into the inner vesicle space. This creates a proton motive force (PMT) within the inner vesicle. The PMT then activates the ATP synthase complex to couple ADP and Pi to form ATP. The pre- cursors ADP and Pi are encapsulated within the inner membrane space. As the machine is carrying its own components to generate ATP and is thus independent of a living cell to work. ATP is the biological molecule that provides energy for many biological energy requiring processes and thus can potentially be adapted to activate and power a number of processes. In the process of imparting the chemical energy of ATP to a biological process the ATP is hydrolysed back to ADP and Pi. With this device as these processes take place within the inter membrane space the ADP and Pi are maintained in an enclosed space and can thus be recycled to produce more ATP – in this case a kinesin motor protein. Kinesin has two

heads which move along a tube in a stepwise manner requiring 1 ATP per step. In the cell the kinesin moves along the tubule; the kinesin is fixed and so the tubule moves in the direction of the steps. The control of the machine is through light; if the light is on, then energy flows through the system, resulting in the movement of the tube, if the light is turned off then the machine stops. Light is a very controllable energy source. Thus, it would be possible to calculate the amount of light energy would need to be delivered to the machine to produce 1 ATP and thus make 1 step, or, if more steps are required, then the corresponding amount of energy needs to be supplied. For repeated use of this machine, the tubule also needs to be able to reverse the movement of the tube. A group have recently genetically modified a kinesin by incorporating a blue light activated switch at the hinge between the stalk and the heads (Nakamura et al. 2014). When activated by blue light this reverses the heads and thus the direction of movement of the kinesin. In this way this machine can be used as a piston.

With regards the mass production of the machine here it is proposed to use droplet microfluidics. This requires a suitable channel design, incorporating repositories for the component molecules, micro-reactors to undertake the controlled self-assembly processes, valves to control the flow of the droplets and components taken from a repository and to a micro-reactor, as well as detectors to regulate the quantities of components required for each assembly stage. Also incorporated into the chip design could be internal diagnostics to analyse the initial components, the assembled components and then the final completed machine.

As mentioned previously, once ATP has been produced then a range of biological molecules and processes can be activated. Many enzymes, motor proteins and active transport mechanism can be controlled. Thus, there is a great number of potential applications that this technology could be applied to.

As described above, once there is such a device, this technology can be applied to manufacture novel materials and devices – for example, in the medical field, such as minimal invasive surgery particularly on tissues or organs that are very delicate. Being sub-cellular in size, such devices could be injected into the body and then directed to the site to undertake the surgical manipulation required. Once this has been completed, being biological, the device can be programmed to degrade and be incorporated into the cellular structure.

The potential application of such a device are:

- A drug delivery system.

- Precision nano-engineering.

- Minimal invasive nano-surgery of delicate tissues like the retina, lungs or alimentary canal.

- Detoxification of polluted environmental sites.

This technology, while in its infancy, can thus achieve major advances in human and environmental well-being. Its use requires not only scientific and technological understanding, but also creativity and imagination in rethinking how we address significant problems faced by humanity.

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