Molecular Machines: The Future of Modern Healthcare

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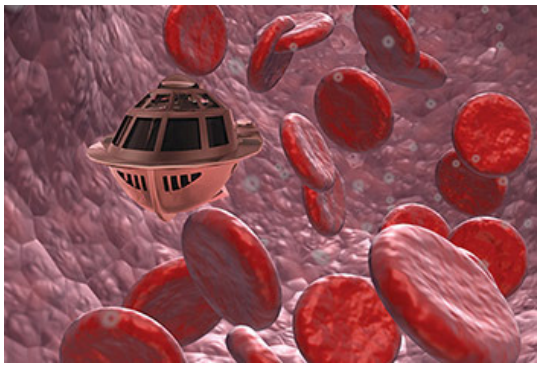

Figure 1 - still from Fantastic Voyage, 1966

Two hundred years ago, engineering was entering a time of immense change. A rapidly growing population meant there was a new demand for efficiency, fuelling the rise of machinery to power the nation. Parallels can be drawn with our world today, with increasing life expectancy and exponential population growth posing new problems for healthcare. Infectious diseases can be diagnosed and treated faster than ever before, but the threat of antibiotic resistance grows stronger with every prescription, while 28% of all UK deaths are still caused by cancer. [1] But within us all is a toolkit of tiny machines, that spin faster than jet engines and can walk like robots. No, this isn't the plot of the 1960s B-Movie 'Fantastic Voyage' (Figure 1 [2]). It isn't science-fiction at all. An army of molecular machines control every aspect of life with speed and precision. By applying these ideas in the laboratory, we are on the brink of a medical revolution. Just like the Industrial Revolution, machines are central to change. Only this time, 50,000 of them could fit along the width of a single human hair. [3]

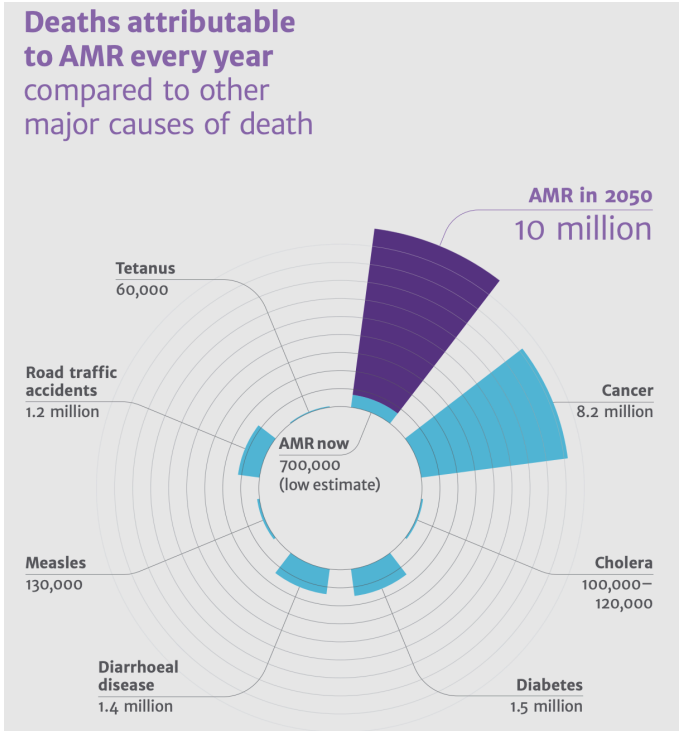

Figure 2 - chart showing threat of AMR in comparison to other causes of death

As Professor of Engineering Paula Hammond of MIT explained in her recent TED talk, "Engineering at the molecular level, working at the smallest of scales, can provide exciting new ways to fight the most aggressive forms of cancer." [4]

And this healthcare revolution couldn't come at a more pivotal moment. As well as cancer, antimicrobial resistance (AMR - including antibiotic, antifungal, antimalarial and antiviral resistance) is set to be a crisis of the coming decades. Every year, at least 700,000 die of drug-resistant diseases, [5] and more are set to become untreatable. By 2050, annual death tolls from AMR are set to reach 10 million, which is even higher than current cancer deaths (Figure 2 [6]). AMR can be caused by misuse and overprescription of antimicrobials [7], whereby microorganisms evolve resistance to a particular drug. This can happen naturally through genetic mutation in some individual microorganisms, but when a

[1] Cancer Research

[2] Fantastic Voyage

[3] WE Forum 09/2017

[4] TED Talk by Professor Paula Hammond of MIT

[5] WHO Antimicrobial Resistance

[6] AMR Review, 2014

[7] CDC Antibiotic Resistance Q&A

mutation spreads through a population of microorganisms, previously treatable diseases become killers. Such a multifaceted problem requires a sophisticated solution, and that is where molecular machines can help.

Combatting Antimicrobial Resistance

Engineered molecules have the potential to physically drill into resistant bacterial cells, allowing antibiotics to become effective again. In December 2019, researchers used molecular nano machines (MNMs) to disrupt bacterial cell walls, making them much less resistant to antibiotics. [8] A team of scientists were able to kill the highly resistant *Klebsiella pneumoniae*, identified by the World Health Organisation as a high priority pathogen. [9] This bacteria can cause pneumonia, bloodstream infections and meningitis, especially in hospital settings. In the team's laboratory experiment, the MNMs were added to a solution of bacteria, and within minutes, they drilled in and killed the bacteria. According to James Tour, one of the main researchers, the bacteria have robust cell walls as opposed to a lipid bilayer like human cell membranes, "But they have no way to defend against a machine like these molecular drills, since this is a mechanical action and not a chemical effect."

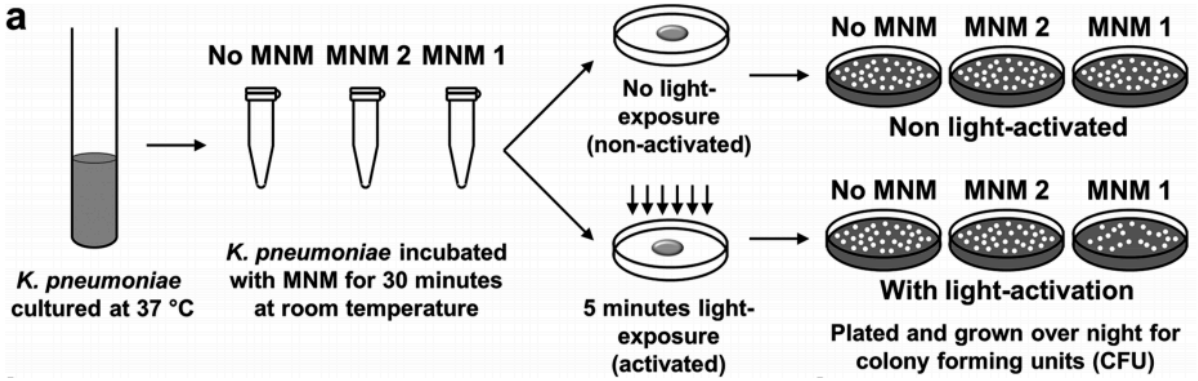

Figure 3 - diagram showing experiment with MNM 1 and MNM 2

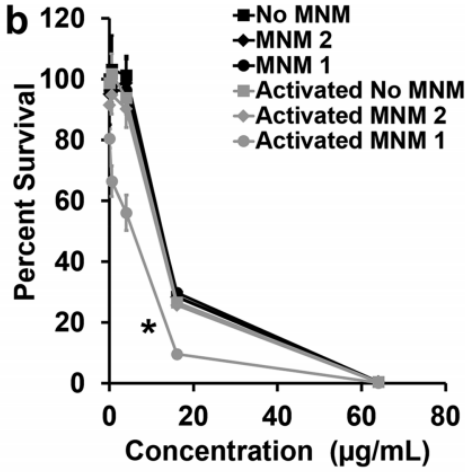

Figure 4 - graph showing percentage of *K. pneumoniae* that survived different concentrations of Meropenem with MNM 1 and 2

The investigation involved different types of MNM; MNM 1 which rotates unidirectionally 2-3 million times per second (faster than a jet engine) when activated with UV light, and MNM 2, a much slower control measure. They compared non light-activated and light-activated samples, (Figure 3) demonstrating that without light, MNMs can diffuse in and out of cells without causing damage. This could prove very effective in targeting specific cells and not causing harm to surrounding areas, operating with high precision, because the MNMs would not be activated until exposed to UV light.

In just five minutes of UV light exposure, there was a 17% decrease in the viability of *K. pneumoniae.* With longer periods of exposure this figure increased, and results were especially promising when MNMs were combined with the drug Meropenem. Usually, *K. pneumoniae* becomes resistant to drugs like Meropenem by losing its cell wall pores, blocking the antibiotic, but MNMs create pores, so the bacteria becomes susceptible to Meropenem again. This graph (Figure 4) shows that increasing the concentration of Meropenem decreases the percentage survival of *K. pneumoniae,* especially for the light-activated MNM 1, where less than 20% of the bacteria survived at a low concentration. The

[8] ACS Nano 2019, 13, 14377-14387

[9] WHO High Priority Pathogens

results of this study show that MNMs have huge potential to target a variety of different bacterial infections, and seriously reduce the threat posed by AMR in the near future.

The mechanism

But how exactly do these molecules work, and how can they be engineered to perform different tasks to revolutionise modern medicine? Different parts of the molecule must be able to move independently for the machine to work. It's all to do with stereochemistry (arrangement of molecules). [10] The nanomachines used in the above study are overcrowded alkenes, organic molecules that display photochemical cis-trans isomerism around the carbon double bond at the centre of the molecule. (Figure 5 [11]). This sounds complex, but it means that when excited by light, the two groups either side of the centre (the rotor and the stator) transform into a different arrangement, changing the shape and producing 360 degree rotation of the motor. Added methyl groups (carbon side chains) prevent it rotating back on itself. Just like a familiar macroscopic motor, this molecule can produce movement, and therefore can be used to drill into cells at a high speed.

Figure 5 - molecular structure of a nanomachine

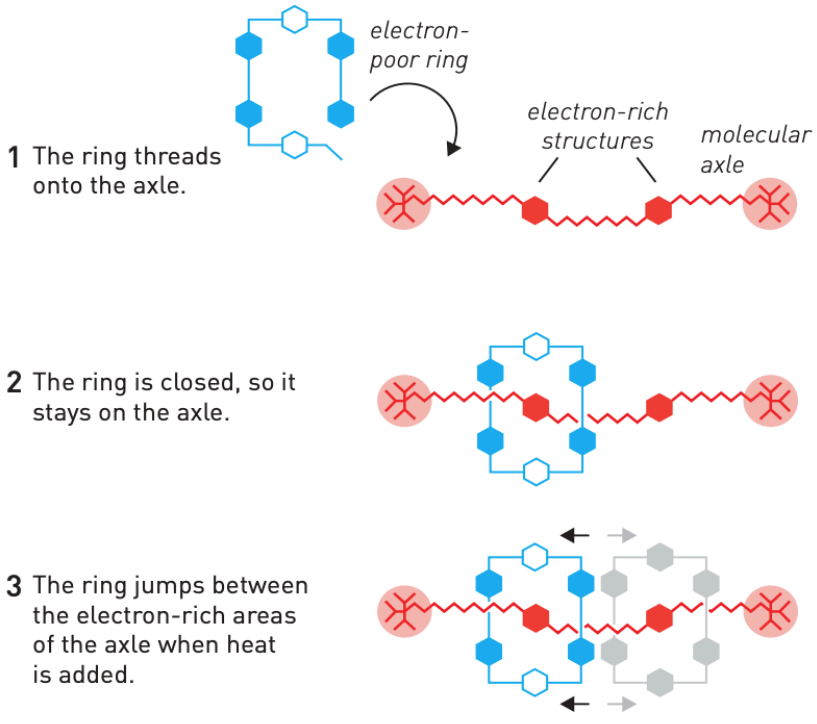

Figure 6 - the molecular mechanism of Stoddart's rotaxane

The complex mechanisms of a toolbox of molecular machines are decades in the making, and are now being refined to be used in healthcare, from cancer treatment, to drug delivery and rapid diagnosis.
In 2016, three scientists, Jean-Pierre Sauvage, Sir J. Fraser Stoddart and Bernard L. Feringa, won the Nobel Prize in Chemistry "for the design and synthesis of molecular machines." [12] This was a culmination of their work going back several decades.

In 1983, Jean-Pierre Sauvage built the first catenane, a new type of molecule which isn't bonded chemically, but mechanically. [13] Unlike in a covalent bond, where electrons are shared between atoms, a mechanical bond is just as the name suggest, with structures mechanically interlocked. Fraser Stoddart

[10] Fessenden and Fessenden Organic Chemistry 3rd Edition 1986, page 117

[11] ACS Nano 2019, 13, 14377-14387

[12] Nobel Prize in Chemistry 2016

[13] Nobel Prize - Jean-Pierre Sauvage

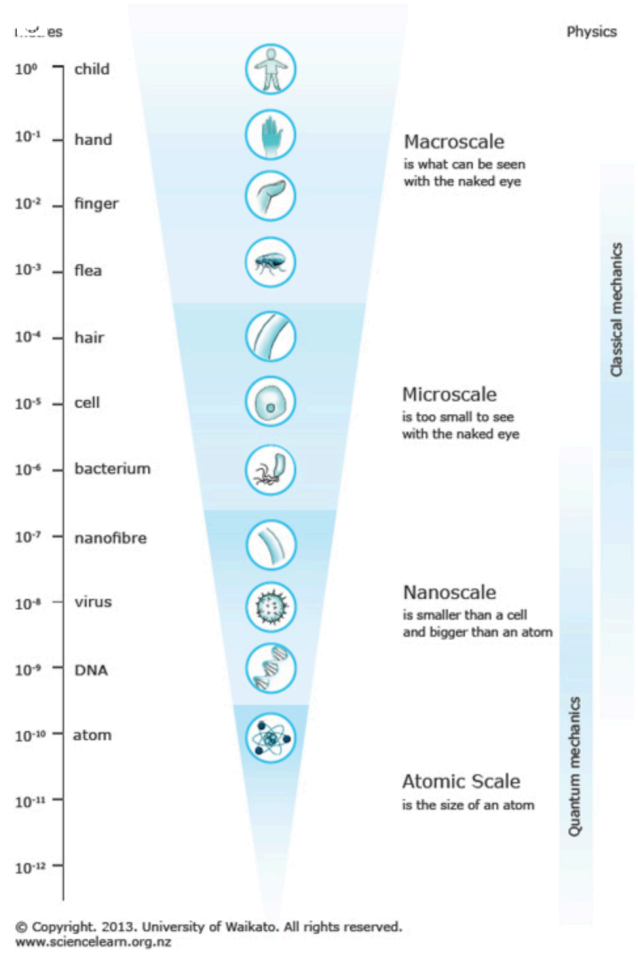

Figure 7 - different scales and the laws governing them

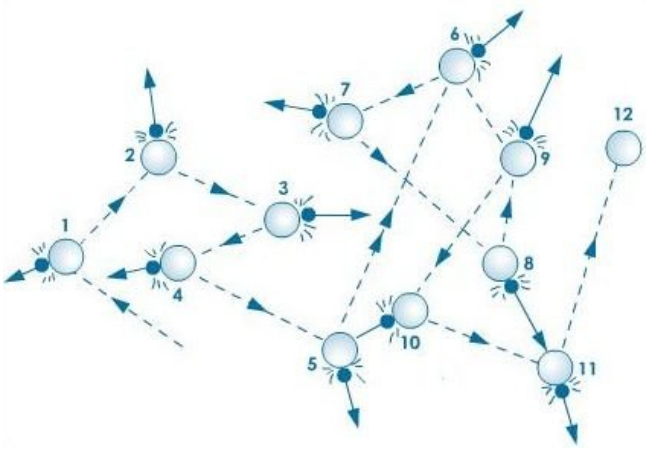

Figure 8 - Brownian motion of particles

took it a step further in 1991, creating the first rotaxane, another new class of molecule featuring a ring mechanically threaded onto an axle.[14] Using the law of electrostatic attraction, a ring lacking electrons is attracted to an axle with points high in electrons, so when in solution, the ring threads onto the axle and can jump between the two electron-rich points, (Figure 6). This was a huge step forward, and Stoddart's team used this rotaxane structure to create molecular lifts, futuristic computer chips and even an artificial muscle fibre.

The first motorised molecule, like the one used in the *K. pneumoniae* experiment, was engineered by Ben Feringa in 1999. Using these motorised molecules, Feringa and his team built a four-wheeled nanocar. The work of these scientists in developing this toolkit of molecular machinery makes the prospect of a healthcare revolution possible, because with further refinement, we could mirror and engineer to enhance the highly specialised biomolecules already keeping us alive every day.

Engineering challenges

But there are many challenges that need to be overcome before nano machines as sophisticated as the ribosome (the site of protein synthesis) [15] can be engineered in the laboratory. Working at the nano level has huge impacts on the properties of different materials. Tiny particles have a much larger surface area to volume ratio, which changes how they behave in chemical reactions. The laws of physics change for nano materials too. Macroscale materials behave according to classical mechanics, governed by set laws relating to forces, however nano materials are influenced by the laws of quantum mechanics, (Figure 7 [16]). At the nanoscale, gravity has much less of an impact because it relies on mass, which becomes negligible at such a small scale. In contrast, Van der Waals forces become far more important, because they are based on electrostatic attraction, unaffected by the mass of the particles. Van der Waals forces are intermolecular and can arise between charged, polar and neutral molecules, due to electron movement causing an instantaneous dipole (charged area) to arise in one part of the atom. This can induce charge in neighbouring molecules, leading to attraction, even in molecules which are electrically neutral overall. Van der Waals forces are important in biological reactions and even more important at the nano scale, where they govern the behaviour of materials. This makes their properties different to the bulk materials used in engineering.

[14] Nobel Prize - Science Background

[15] Biochemistry, 3rd Edition, Lubert Stryer, 1988

[16] Nanoscale properties

Brownian motion (random movement of particles in a fluid) is also important, (Figure 8 [17]) because on such a small scale, the movement of individual particles has a greater effect. Achieving directionality in molecular machines is a challenge. Molecular drills as used in the antibiotic resistance experiment, for example, must be able to rotate at least 3 million times per second to "overcome obstacles presented by adjacent molecules and outpace natural Brownian motion," [18] according to researchers at Rice University. All of these difficulties posed by the physics of nanotechnology, combined with the fact that trials for developing new treatments are costly, means that it could be a while before we see engineered molecular machines working in the NHS.

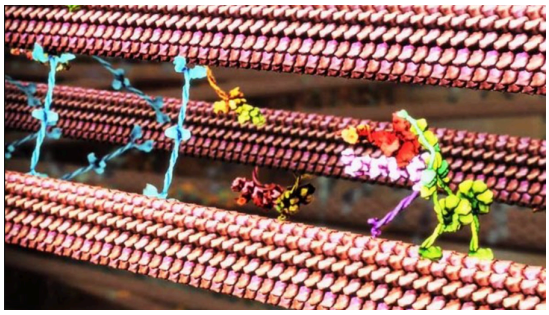

Figure 9 - bio-molecular machines along microtubules

Modelling biomolecules

However, some scientists believe the way to improve the rate of progress in this field would be to model molecular nanomachines on the complex biomolecules already at work in our bodies. This philosophy, biomimetics [19], looks to machines already well evolved to work on the nanoscale, using just the 20 amino acids as building blocks. Due to the difference in the physics of nanotechnology, technomimetics (scaling down of macroscale mechanics) often doesn't work. According to physicist Dean Astumian, University of Maine, "The entire regime of motion in the molecular world is completely different to in the macroscopic world, and so what people call nanocars have nothing at all to do with the physics of a car." [20] Another example is David Leigh's two legged motor which 'walks' down a track. The motion is unrecognisable from robotics, using chemical reactions to secure the legs as gravity is negligible. It uses acid-base chemistry, pH oscillation, to move the molecule, and uses the same photochemistry principles as the molecular drill to maintain direction. Leigh and his team modelled this walking nanorobot on proteins in the body. Molecular motors carrying proteins walk along spindle fibres during cell division (Figure 9 [21]), and this biological mechanism could be replicated by engineers in order to build more sophisticated machines. Leigh intends to use these mechanisms to construct robotic arms to pick up amino acids. His team recently engineered a machine mimicking the ribosome, which can build peptide chains that could be used to assemble proteins. With refinement, it could be used to create enzymes to catalyse a variety of reactions, which could be applied to all areas of medicine.

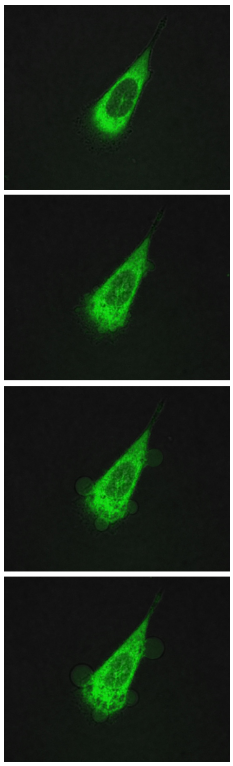

Figure 10 - Images from Durham University study - blebbing in prostate cancer cell membranes over 10 minutes

Applications in cancer treatment

When molecular machines have been refined to be used in healthcare, the impact they could have is massive. They are already showing promising results in the fight against cancer. At Durham University in 2017, researchers used motorised molecules with peptide addends to recognise and bind to human prostate cancer cells [22]. Within 3 minutes of UV light activation, the molecules had killed the cancer cells. The research team tagged cells with green fluorescent proteins to capture images of blebbing, (Figure 10) where the membranes bubbled and cytoplasm spilled out, showing that the cell had died through necrosis (death of a cell through injury). This mechanism could

[17] Brownian Motion

[18] Rice University - molecular motors

[19] PNAS - Molecular Machines with Bio-inspired Mechanisms

[20] Chemistry World - Molecular Machines

[21] Drew Berry animations

[22] Rice University - Motorized Molecules

help to make cancer treatment less invasive, particularly as the machines diffuse in and out of cells easily and don't rotate until UV light activation. However, to make them more suitable for use, they would need to be activated with lower frequency light, as UV light can ionise surrounding cells, causing damage to healthy tissues. Researchers do believe this will be possible, by two-photon absorption, a technique with similar frequencies to infrared light, which would be safer. [23] Once optimised for *in vivo* use, the peptide addends could be adapted to adhere to different cancers, such as breast cancer, and so the effects of this treatment could be far reaching. Another use could be targeted drug delivery; the pores made by the nano machines could deliver drugs to specific cells, again useful in cancer treatment. With more trials, future generations could benefit from nanomachines to alleviate a wide range of different diseases.

With this medical revolution, the doctors' mantra 'primum non nocere,' first do no harm, may actually become achievable. With recent figures released by NICE showing some 300,000 patients contract infections from NHS healthcare every year [24], it is clear that hospitals sometimes do more harm than good. Molecular nanomachines could prolong the usage of current antibiotics, saving lives and stretched NHS resources. It could also make cancer treatment less invasive, more effective and tailored to the needs of the patient. Medical nanotechnology is in its infancy, however the idea of using nanoscale materials has already been successfully used in Diabetes patients, with the introduction of bandages incorporating silver nanoparticles with antibacterial properties, to speed up the healing process. [25] As Richard Feynman said in his 1959 lecture "There's Plenty of Room at the Bottom," when predicting the future development of nanotechnology, "I would like to describe a field, in which little has been done, but in which an enormous amount can be done." [26] The potential to revolutionise medicine with the engineering of molecular machines is endless.

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[23] TMC - Motorised Molecules

[24] NICE - Infection Prevention and Cure

[25] Vita Scientific - Nanotechnology in Medicine

[26] Richard Feynman - There's Plenty of Room at the Bottom, 1959

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