"Be More Positive: A New Method for Evolving Proteins in the Lab"

Our modern day life is filled with benefits stemming from protein-based technology. Proteins are biological substances constructed from a chain of 20 different building blocks called amino acids. These building blocks can be arranged in many different ways, resulting in a wide variety of proteins. In fact, there are around 2 million proteins with diverse functions that compose all life. Besides their biological importance, proteins have a second, secret life: Outside of our bodies, our society is surrounded by protein technology. For instance, protein in laundry detergent helps ease the washing process by breaking down the dirt on clothes. Proteins are also extensively used in medicine, beginning from the very first protein drug - human insulin introduced 35 years ago for the treatment of diabetes. Pharmaceutical companies have since been eager to develop a variety of protein-based drugs, particularly in cancer therapy because of their relatively low side effects and ability to target tumour-bearing tissues. Indeed, there are currently more than 200 protein drugs that have been approved by the US Food and Drug Administration.

Proteins are not without their problems however: Two major issues are insolubility and cell impermeability. The inability to dissolve in water is caused by the proteins sticking together to form a large mass, leading to their dysfunction and difficulty to deliver to the blood stream or through an injection needle. The inability to enter cells is caused by the physical barrier of cell surfaces, limiting the medical effectiveness of protein drugs. Supercharging emerged as a method to overcome these problems by endowing proteins with exceptionally high electric charge on their surfaces. Because like charges repel each other, supercharged proteins no longer clump together and insolubility issues are dramatically improved. Another remarkable benefit of positively supercharged proteins is the increased efficiency with which they can enter cells, having an effect such as, potentially, killing the cell. These attractive features thus have taken many scientist's interests in engineering of supercharged proteins.

How can supercharged proteins be designed? Unfortunately, no ideal or general blueprint has been established to date. We are very keen to solve this scientific problem using a modern biotechnological approach called "directed evolution". The 19th century naturalist, Charles Darwin, postulated that all life forms on Earth have evolved from their ancient forms to what they are today through repeated random mutations and natural selection over billions of years. For instance, giraffes have acquired long necks through a long process whereby giraffes with slightly longer necks were more successful in reproducing and so passed along this trait to their offspring. This is a natural selection process. Although natural evolution is slow and gradual, such processes can now be mimicked to evolve proteins in the laboratory in a much shorter time scale. As proved by the three pioneers winning the Nobel Prize in Chemistry in 2018, this evolutionary approach is extensively used for instilling user-defined functions into proteins.

We are proposing a new method to evolve proteins with positively supercharged characteristics using a unique nano-sized protein cage. This nanocage has a negatively supercharged interior and so can accommodate positively charged guests. Based on the nano-packaging method, we have built a system in which only positively supercharged proteins can enter the cage and be protected from being destroyed, while non-positively supercharged proteins cannot be encapsulated and are destroyed. This provides an artificial selection process: Any protein with a high positive charges will have an advantage to survive in this artificial selection pressure, giving us a great chance to evolve a suite of positively supercharged proteins.

In summary, despite their potential, there is a significant number of proteins that have failed for therapeutic usage due to strong aggregation or poor cellular uptake. Protein supercharging is one of the most promising strategies to surmount these practical issues. We anticipate that our unique evolutionary method for engineering supercharged proteins will introduce new tools and drugs for medicine and biotechnology.