Production of Troublesome Proteins *in Capso*

Proteins are familiar to many of us; they are the main constituents of all living things. Our own cells are packed full of proteins that carry out many tasks essential for our survival. Not surprisingly, when proteins stop working correctly, diseases result. For this reason, many different proteins have been the subject of intensive research throughout the world to understand how they work. Apart from their natural roles in living things, proteins also form the basis of many important technologies. For example, they are used as moisturizers for cosmetics, antimicrobial finishing agents, as well as detergent- and food additives. Some proteins are also important medicines, particularly in cancer therapy because of their relatively few side effects and ability selectively attack tumors. To date, more than 200 protein drugs have been approved by the US Food and Drug Administration.

With the high demand for proteins, where can we get them? It depends on the protein. Sometimes, they can be taken from the source organisms: an apple-derived protein is directly isolated from apples. Unfortunately, things are not always that simple. Useful proteins may be in disease-causing organisms that would be difficult to grow safely or may be produced in only tiny amounts in their natural environment. The solution to this problem came about in the 1970s when researchers found a way of manipulating simple, safe and fast-growing bacteria to produce required proteins that are originated from different organisms. Since then, the use of the "micro-factories" has blossomed as a safe, easy, scalable, time- and cost-saving means for protein production.

The breakthrough method using bacteria has not yet solved all problems in protein production. There are important proteins that bacteria find very difficult to produce. For example, a protein antibiotic obviously can't be produced by bacteria – they'd kill themselves! Not only that, but some of the most important treatments for human diseases consist of replacing malfunctioning human portions with the corrected versions. Unfortunately, many human proteins are not tolerated by bacteria. They are often quickly destroyed or degraded soon after produced and before harvested.

How can we solve this problem and get bacteria to produce these important proteins that they seem to dislike? The answer is to trick bacteria as if the proteins are *not there at all*! Our idea is to produce nano-sized containers in the bacteria. We engineer the system so that as soon as our difficult-to-produce proteins are made by the bacteria, they spontaneously enter the nanocontainers. The bacteria then have no way of sensing what is in them or of destroying their contents. Our nanocontainers are also made of proteins, called "capsids", from the Latin *capsus* meaning cage/carriage, and we named our technology to package other proteins inside them "encapsidic production". Our preliminary studies showed that a fragile protein that is normally destroyed rapidly in bacterial cells was protected by being inside the capsids and therefore could be purified in a functional form.

The goal of the research is now to develop the potential of this system as a new general biotechnology for obtaining proteins that have been difficult to generate with conventional methods. There are many such potentially useful proteins and we will optimize our capsid to production for such proteins. To the goal, we will use an approach called directed evolution that mimics naturally-occurring evolution process but in the laboratory and on much shorter time scales. At the same time, we will try to develop new ways to open up our capsids to retrieve the desired proteins. We anticipate that our unique capsid-based biotechnology will largely support development of protein related sciences, and perhaps ultimately helping to produce new protein medicines.