# Protein scissors that also learned to glue

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**An enzyme found in plants has some remarkable abilities that have drug designers excited.**

Artwork by Scot Nicholls, Domokun Design, reproduced from Chemistry & Biology

Proteins are a hotly researched area of drug design, but proteins can be degraded by the human body. Protein rings, however, are super-stable, giving them greater potential as drugs.

The main way to make protein rings is by chemical synthesis, but this is costly, inefficient and uses toxic chemicals. However, we have worked out how sunflowers turn a protein string into a super-stable protein ring in their seeds.

The enzyme that performs this ring-forming reaction is one that usually cuts proteins, but instead of using water to finish the job it uses the head of a protein chain to form a new bond instead of breaking one. The ability to develop water-based reactions will eliminate the need for expensive and harmful chemical synthesis of drugs that are stable protein rings.

The work on this unusual ring-forming reaction began with a small protein found in the seeds of sunflowers called SunFlower Trypsin Inhibitor 1. SFTI-1 is a ring of 14 amino acids that has no head or tail.

SFTI-1 was discovered in the late 1990s and became popular with synthetic protein drug designers, but only in 2011 did the

gene that encodes SFTI-1 become known. The sequence for SFTI-1 is buried inside a gene that also makes seed storage albumin, a protein that accumulates to very high levels in seeds before degrading during germination to provide nutrients for the growing seedling.

How the sequence for SFTI-1 came to be buried alongside an albumin seemed curious indeed, but recent work has found that sequences for protein rings like SFTI-1 have been buried in albumin genes for at least 28 million years. SFTI-1 is just the first known member of a very large family of tiny seed proteins.

Seed storage proteins were worked on a lot in the 1990s. Ikuko Hara-Nishimura and her Japanese colleagues found a protease called AEP that helps to assemble seed storage proteins. It transpires that the albumin and its adjacent SFTI-1 both need AEP to be assembled correctly.

A breakthrough that made it possible to study the "string to ring" reaction was the ability to make AEP properly in bacteria. *Escherichia coli* has been used in labs all around the world to make tens of thousands of different proteins, but it sometimes struggles to make certain proteins. Some proteins are toxic to *E. coli* whereas others don't fold properly and, once extracted, are insoluble in water. Sometimes a protein can be soluble and correctly folded, but *E. coli* cannot decorate the protein with the right chemical modifications for it to be active.

Over the years, the number of engineered *E. coli* strains has continued to grow, each with different capabilities. The *E. coli* strain that enabled us to make active AEP was a new strain that was especially good at protein folding and forming sulphur–sulphur bonds, which isn't something *E. coli* usually does well.

We used this new strain of *E. coli* to make and purify a suite of AEPs. Once we had pure AEP we changed the pH and the AEPs would self-activate by cutting themselves in several places. This self-activation by proteases is quite common.

We mixed these activated AEPs from *E. coli* with a range of protein strings containing the sequence for SFTI-1, and found that AEP uses a rather ingenious way to make a protein ring.

Proteases usually cut proteins for two reasons: enthalpy and entropy. Enthalpy refers to the energy balance of the reaction. Cutting a protein releases energy whereas forming or ligating a protein bond usually requires an energy input. Hence bond-breaking is easier to do than making one.

Entropy is about order. Cutting a protein usually gives you two proteins. It's easier for one piece of protein to be cut into two than for two disconnected proteins floating in solution to come close enough together to be joined by an enzyme into one.

For SFTI-1 to be made by a protease, these barriers of enthalpy and entropy must be overcome.

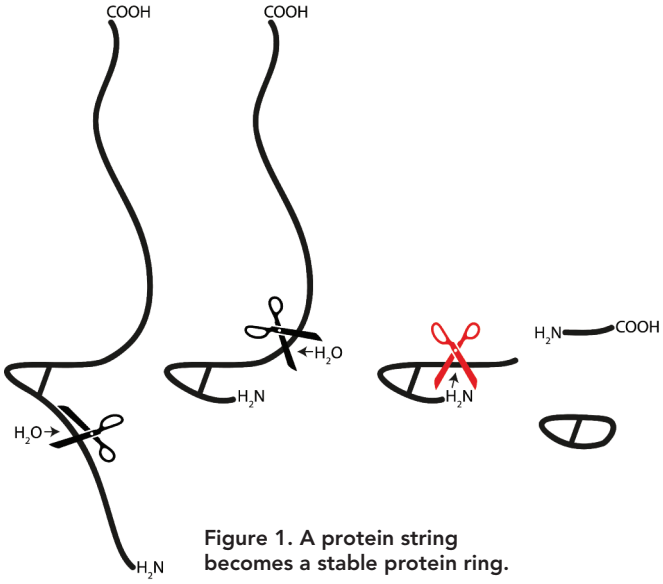


**Figure 1. A protein string becomes a stable protein ring.**

Proteases usually cut protein bonds by binding to the protein and then using water to hydrolyse the bond. However, water isn't involved when SFTI-1 is made.

The SFTI-1 string is made into a ring by cleaving off and discarding a small part of the string and using a nearby amino head group ($H_2N$) instead of water ($H_2O$) to complete the reaction (Fig. 1). To overcome enthalpy and entropy, AEP uses the energy obtained from cleaving to join the head of SFTI-1 to its tail in a reaction that moves a protein bond from one place to another place that's held really close by. The result is a circular protein with no ends.

During the same studies we found that if SFTI-1 did not get made into a ring it quickly broke down. Sealing the ends of a protein together makes it more stable, and this process has the potential to be industrialised using AEP. The ability to seal off the ends of a protein by joining them together is therefore a popular approach that protein drug designers use to stabilise proteins that would otherwise be rapidly broken down in biological fluids.

An interesting finding from our work, which has been published in *Chemistry & Biology* (tinyurl.com/j5w4sk9), was that only one of four AEPs could perform the ring-forming reaction to make SFTI-1. The other AEPs could only cut the string into two pieces of string. What was perplexing is that this bond-making AEP looked very similar to the bond-breaking AEPs.

A different ring-forming enzyme in cyanobacteria has an extra protein "wing" that helps protect the active centre of the enzyme from water, so how our bond-making AEP keeps water away from its centre must be more subtle.

Future work will attempt to understand what changes in the genetic sequence turn a cutting AEP into a ligating AEP and what changes improve its ligating ability. This should allow the engineering of artificial enzymes with superior cutting or ligating ability for industrial applications in biotechnology and protein drug stabilisation.

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