Engineering Proteins for the Prevention of Disease Progression

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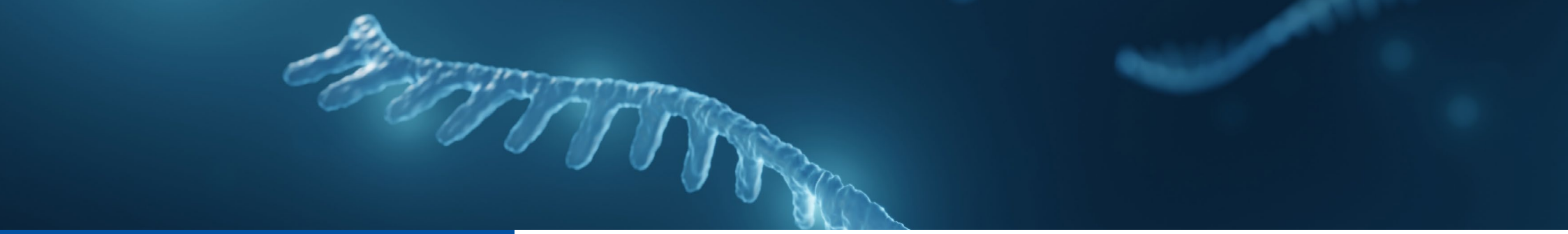


Engineering Proteins for the Prevention of Disease Progression

The way in which viruses invade and replicate within their hosts involves a multilayered system of protein-based interactions, and understanding the mechanisms at play is crucial when developing potential treatments. Utilising new techniques such as genetic code expansion, Professor John Paul Pezacki and his team of researchers at the University of Ottawa in Canada have designed a novel, highly specific artificial protein complex which can halt the progression of viral infections in human cells. They have identified and described a novel approach to wider preventative and restorative therapeutics in human disease.

Target: The Nanomachines

Enzymes play an important role in biological function, since they are responsible for facilitating a variety of molecular reactions within the body, and they may be specific or non-specific in their actions. Endonucleases are a type of enzyme which help to break the bonds linking the molecules within genetic structures, and it is this function that assists in repairing DNA and dampening the response to viral infections. It is possible to alter the functionality of naturally occurring endonucleases so that they are directed towards specific targets to degrade or suppress very small DNA-related proteins called micro-RNAs (miRNAs).

miRNAs were discovered and described by Amercian scientists Victor Ambros and Gary Ruvkun, leading to their award of the Nobel Prize in Physiology or Medicine in 2024. Although discovered in nematode worms, miRNAs are now being looked at in the context of disease, given their vital regulatory roles in gene expression, cellular metabolism, and immunity.

No naturally occurring endonuclease is capable of specifically promoting the degradation of miRNAs. Although some advancements in the development of functionally adapted endonucleases have been achieved in recent years, researchers have faced challenges due to the inability to attain a suitably unique final product which supports the ongoing activity of the manufactured enzyme.

Genetic code expansion is a new and powerful technique that allows researchers to incorporate amino acids into proteins. Professor John Paul Pezacki and colleagues in the Department of Chemistry and Biomolecular Sciences at the University of Ottawa in Ontario, Canada, used this approach to engineer an entirely new endonuclease capable of precisely homing in on target miRNAs, with a view to preventing the advancement of disease in humans, including the hepatitis C virus and various cancers.

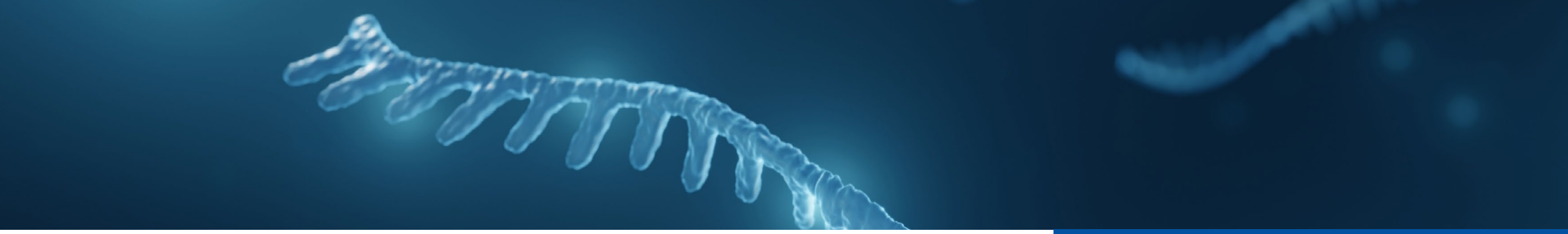
At the time of writing, other work in the group is directed at targetting other types of RNA molecules, including genomic material.

Creating a Killer

To achieve this, Professor Pezacki and team first had to select a suitable protein to form the basis of their enzyme product, and settled on the highly selective viral suppressor p19, a potent inhibitor of small RNA function. They deduced that p19 is an ideal candidate for further engineering to unlock 'super suppressor' powers in combination with endonuclease activity. Certainly, a low affinity for larger DNA strands, and the ability to retain its binding functionality makes p19 particularly appealing for this purpose. Previously acquired knowledge regarding the expansion of the genetic code and the binding of unnatural amino acids to metal-based molecules demonstrates that these bonded complexes can be incorporated into specific locations in the protein of interest to induce RNA cleavage. Therefore, the researchers decided to explore whether introducing a copper-bound artificial amino acid into a specific site on p19 could impart endonuclease activity, and what impact this might have on the cleavage of miRNAs in human cells and the subsequent progression of disease.

Optimisation and Testing

A catalytic site was inserted in the RNA binding area of p19, and the optimal position for the introduction of the selected artificial amino acid-metal complex was identified using information gained from previous experiments investigating RNA cleavage. Incorporation of the enzyme into p19 and its affinity for specifically binding and efficiently cleaving small RNAs was confirmed using molecular-level analytical techniques.



Once the specificities of the artificial enzyme-metal complex had been determined, the potential endonuclease activity in human liver cancer cells was investigated. Professor Pezacki and his research team discovered that their engineered enzyme could isolate and degrade a specific, abundantly present liver miRNA known to be a significant promoter of hepatitis C virus replication; indeed, this miRNA stabilises the virus and supports its progression. The hepatitis C virus is a causative agent of liver and lymphatic cancers and liver disease. Crucially, when the experiments were repeated with the naturally occurring, non-engineered p19 protein, the selected miRNA fragments could be isolated but not destroyed.

With this in mind, Professor Pezacki and the team proceeded to investigate the effect of their novel endonuclease on the replication of the hepatitis C virus. They used a disabled virus system known as a replicon to validate the antiviral role of their engineered enzyme. They discovered that it inhibited the hepatitis C virus in a similar way to established miRNA silencers called antagomirs, which are commonly used to stop disease progression. They deduced that their endonuclease complex isolated and reduced the amount of miRNA present in the infected cell, thus preventing the binding of the miRNA by the virus and halting its replication.

Target: Destroyed

Professor Pezacki's team concluded that their artificial enzyme could be successfully inserted into a chosen protein to specifically target and cleave hepatitis C virus miRNAs and also exerts a similar effect in human liver cancer cells. They demonstrate for the first time that it is possible to engineer sleek, targeted enzymes with enhanced endonuclease capacity without interfering with structural or functional features. This has been achieved by translating the information encoded within the genetic material and adding metallic molecules that bind to the proteins produced to impede native interactions, building upon the recent trend of using catalytic enzymes to inhibit viral infections. The researchers have revealed a novel therapeutic angle in which disease progression can be hindered by introducing the engineered enzymes with their supplemental functionality into specific disease-related proteins to interfere with binding activity and prevent viral and cellular replication. Furthermore, they have identified a feasible alternative to antagomir-based RNA therapies, which are expensive and difficult to implement, somewhat diminishing their effectiveness. Finally, unlike other techniques, this catalytic approach can destroy many targets.

More in-depth structure-function analyses are certainly necessary to delve into the possibility that the artificial endonuclease created by Professor Pezacki and colleagues can preferentially bind and cleave the miRNAs most commonly seen in the liver. Moreover, future iterations may mitigate any issues surrounding specificity and potency by identifying the individual miRNAs which facilitate preferential binding and cleavage in other disease states.

These crucial insights have huge implications in the fight against viruses such as hepatitis C and other conditions, including cancers of the liver and immune system – and much more. Professor Pezacki's lab is also working to target other molecules, including genomic material of pathogenic organisms such as viruses and bacteria. The ongoing work of Professor Pezacki and his esteemed team of researchers will undoubtedly pave the way for disease treatments of ever-increasing sophistication, providing relief and hope for millions of patients and their families.

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MEET THE RESEARCHER

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Professor John Paul Pezacki, from the Department of Chemistry and Biomolecular Sciences, is the founding director of the Centre for Chemical and Synthetic Biology, and currently holds several cross-departmental appointments at the University of Ottawa. From 2023 to 2024, he held the Fulbright Canada Research Chair in the Fulbright Scholars Program. Following completion of his PhD in Chemistry at McMaster University, Ontario, Professor Pezacki was a Senior Research Officer, Group Leader, and Scientific Head at the National Research Council of Canada.

His current research focuses on the interrogation of host-virus interactions to aid in the discovery of novel therapeutic interventions and diagnostic tools, and he has identified several important host factors that restrict virus replication during his laudable research career. Professor Pezacki is the recipient of many prestigious awards, including the Rutherford Memorial Medal for Chemistry from the Royal Society of Canada and the Queen Elizabeth II Diamond Jubilee award for contributions to Canada, and he has authored or co-authored ~200 peer-reviewed publications.

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