A new way to deliver mRNA genomes: Nucleocapsids with evolutionary properties

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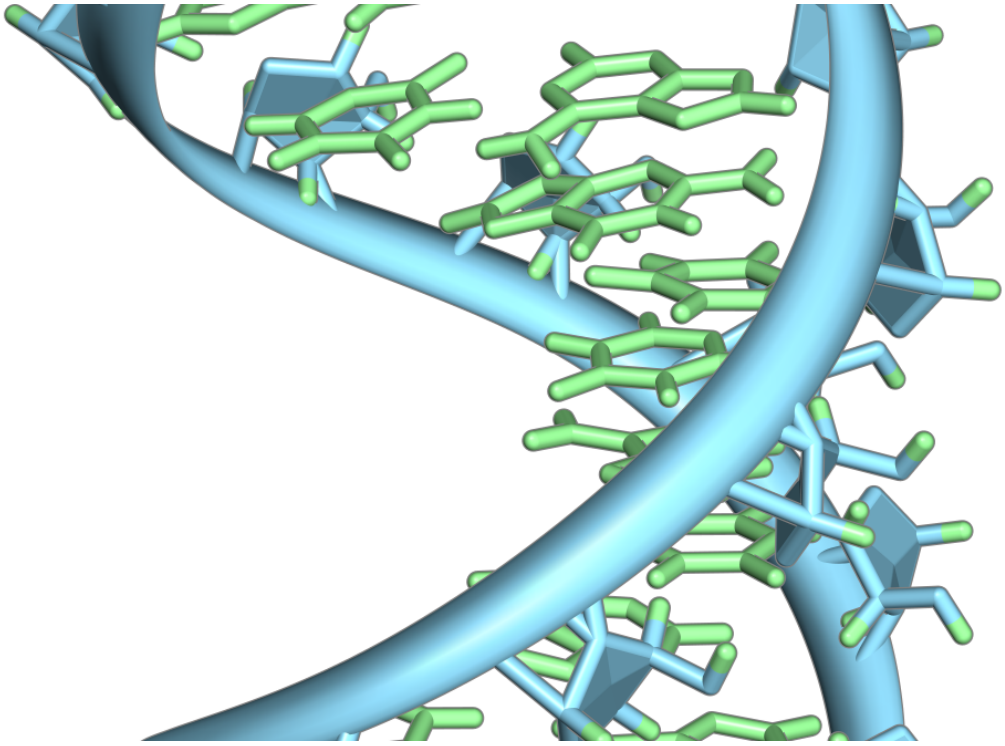

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

(Phys.org)—A team of researchers at the University of Washington has created microscopic assemblies for packaging genetic material that they call synthetic nucleocapsids. The team hopes the assemblies can one day

be used to treat patients with cellular-level problems by delivering appropriate therapies to the cells that could benefit from them. In their paper published in the journal *Nature*, the team describes their assemblies and what they have done with them thus far.

The development of the assemblies was part of an overall program dedicated to mRNA delivery. mRNA, the team explains, are the molecules that cells use to send messages between DNA (the blueprint) and the proteins that reside in the cells. The idea for the nucleocapsids came from viruses, the group further explains, which encapsulate their payloads as a means of protecting them. In designing their own delivery system, the researchers used proteins to build assemblies containing their own RNA genome. The assemblies can evolve indirectly, the team notes—assemblies are created and then injected into test mice. After a period of time has elapsed, blood is drawn from the test mice and examined to see how well the assembly delivered its load to targeted cells. Changes can then be made to improve desirable characteristics.

The researchers note that the technique allows for large leaps in improvement after just a few iterations. They found, for example, that they could improve the survival rate of an RNA package injected into a mouse after six hours from 3.7 percent to 71 percent. They also found that they could slow circulation time inside of a mouse, from five minutes to four and a half hours, giving an assembly more time to do its job.

The will continue to work with the synthetic nucleocapsids, looking for ways not only to improve performance, but to allow for targeting specific types of cells and carrying a wider range of payloads—including RNA, DNA, and proteins and small-molecule drugs. They are hoping the end product will be a delivery system that can be used to battle cancer, heart disease, and other ailments that involve problems with cells.

More information: Gabriel L. Butterfield et al. Evolution of a designed protein assembly encapsulating its own RNA genome, *Nature* (2017). DOI: 10.1038/nature25157

Abstract

The challenges of evolution in a complex biochemical environment, coupling genotype to phenotype and protecting the genetic material, are solved elegantly in biological systems by the encapsulation of nucleic acids. In the simplest examples, viruses use capsids to surround their genomes. Although these naturally occurring systems have been modified to change their tropism and to display proteins or peptides, billions of years of evolution have favoured efficiency at the expense of modularity, making viral capsids difficult to engineer. Synthetic systems composed of non-viral proteins could provide a 'blank slate' to evolve desired properties for drug delivery and other biomedical applications, while avoiding the safety risks and engineering challenges associated with viruses. Here we create synthetic nucleocapsids, which are computationally designed icosahedral protein assemblies with positively charged inner surfaces that can package their own full-length mRNA genomes. We explore the ability of these nucleocapsids to evolve virus-like properties by generating diversified populations using Escherichia coli as an expression host. Several generations of evolution resulted in markedly improved genome packaging (more than 133-fold), stability in blood (from less than 3.7% to 71% of packaged RNA protected after 6 hours of treatment), and in vivo circulation time (from less than 5 minutes to approximately 4.5 hours). The resulting synthetic nucleocapsids package one full-length RNA genome for every 11 icosahedral assemblies, similar to the best recombinant adeno-associated virus vectors. Our results show that there are simple evolutionary paths through which protein assemblies can acquire virus-like genome packaging and protection. Considerable effort has been directed at 'top-down' modification of viruses to be safe and effective for drug delivery and vaccine applications; the ability to design synthetic nanomaterials

computationally and to optimize them through evolution now enables a complementary 'bottom-up' approach with considerable advantages in programmability and control.

Press release