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Lego blocks for precise gene editing

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DNA is the core-element of life as we know it. It can be imagined as a long helical double strand composed of sequences of information written with four chemical "letters" called nucleotides. Determinate sequences of letters delineate stretches of DNA called genes, which in turn are used by the cell as references to decode the information kept within the DNA itself. Gene editing, precisely the molecular act of modifying these DNA sequences, has the potential to revolutionize the field of personalized medicine. Many complex health issues such as Amyotrophic Lateral Sclerosis, obesity and Alzheimer's disease have a genetic component. The ability to correct these abnormalities could greatly increase the quality of life for numerous patients and provide a one-time treatment paradigm for patients rather than a lifetime of drug prescriptions.

Genome editing typically initiates through the creation of a break within the DNA strand. Once the strand is broken, it can then be repaired by the cellular mechanisms through two main processes called non-homolgous end joining (NHEJ) and homology directed repair (HDR). In the majority of cases, NHEJ is used by the cell to repair the breaks. In NHEJ, the strands are reattached in whatever way possible by adding and subtracting bits of DNA until a match is made. Being a trial-and-error mechanism, NHEJ may alter the original sequence because it causes new, undesirable mutations in the genome. Undesirable mutations may lead to a whole panel of challenges for the cellular environment, ultimately leading in some cases to disease formation (*e.g.* cancer).

The second repair mechanism, HDR, uses a precise fragment of DNA as a molecular glue to tie the broken strands together and act as a template to precisely correct (or modify in the case of gene editing) the genome with the desired sequence. This process is often referred to as genome surgery. The possibility of favoring HDR over NHEJ carries major implications for the adoption of genome editing therapeutics in the clinic.

Recent technical advances in the field of genome editing use the breakthrough technique called CRISPR/Cas9. In this system, two components are required for editing: a single strand of RNA called a "short guide RNA" (sgRNA) used to find the gene to be edited within the DNA sequence, and a protein component, Cas9, which functions as a pair of molecular scissors. In order to promote the genome surgery, a donor strand of DNA, which will be used as a template during the HDR, needs to be delivered as well. While the two CRISPR components (Cas9 and sgRNA) can be combined to form one larger complex outside the cell, the donor/template DNA strand must be transported into the cell and localized to the break site independently.

In order to promote a precise genome surgery (HDR), we reasoned that it might be advantageous to create a system where we could attach the genome editing machinery (Cas9 and sgRNA) and the donor DNA together like a set of Legos. Thus, all the materials needed for the gene editing would be brought into the cell simultaneously and localized on the gene to be edited.

To build this Lego set we first modified the sgRNA component of the CRISPR machinery. This modification enabled us to snap in a second

protein, streptavidin, and form a bigger complex that would serve as our central building block. We chose streptavidin since this protein is capable of binding any molecule that is attached to another molecule called biotin (also known as vitamin B7). Biotin is a commonly used molecular modification for many different biological compounds such as DNA, other proteins, or fluorescent tags. Thanks to the versatile properties of streptavidin and biotin, we can pair a Cas9-streptavidin complex with any "biotinylated" molecule and build a larger gene editing complex, snapping in components as needed.

In these experiments, we attached a biotin molecule to the template DNA to tether it directly to the genome editing machinery. After introducing this complex into cells, we found that we increased the ratio of precise genome surgery to imprecise editing on average 10-fold and reversed the balance between these two processes. We also attached fluorescent tags to the complex that enabled the selection of successful genome editing within a population of cells.

In summary, we have been able to create a Lego-like tool where a variety of different components can be attached to the genome editing machinery in order to promote precise genome surgery. With further development, this tool may help us overcome some of the barriers to introducing genome editing in the clinic and lead to a world of brand new therapeutics.