**Lego blocks for precise gene editing**

Gene editing has the potential to revolutionize the field of personalized medicine. Many complex health issues such as ALS, obesity and Alzheimer’s 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 life-time of drug prescriptions.

Genome editing typically functions through the creation of a break within the DNA strand. Repair of this break is accomplished by the cell through two main mechanisms: non-homolgous end joining (NHEJ) and more rarely, homology directed repair (HDR). In the majority of cases, NHEJ is used 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. This process causes new, undesirable mutations in the genome.

The second repair mechanism, HDR, uses a second piece of DNA as a molecular glue to tie the DNA together and act as a template to precisely correct the genome to the desired sequence. This process is often referred to as genome surgery. The balancing act between these two processes carries major implications for the adoption of genome editing therapeutics in the clinic.

Recent technical advances in the field of genome editing use a CRISPR-based system, the most popular of which relies on a specialized protein called 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 a cell, and a protein component, Cas9, which functions as a pair of molecular scissors. In order to promote genome surgery, a donor strand of DNA needs to be delivered as well. While the two CRISPR components (Cas9 and sgRNA) combine to form one larger complex outside the cell, the donor DNA strand necessary to initiate the desired precise repair must be transported into the cell and localized to the damage site independently.

In order to shift the balance of editing away from imprecise edits and instead towards precise genome surgery, we reasoned that it might be advantageous to create a system where we could attach the genome editing machinery, namely Cas9, the sgRNA, and the donor DNA together like a set of Legos. Thus, all needed materials would be brought into the cell simultaneously and localized in the same space.

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 complex we called a S1mplex that would serve as our central building block. In addition to binding to the RNA, streptavidin is capable to binding any molecule that is attached to biotin, a commonly occurring compound also known as vitamin B7. Biotin is a common molecular modification and has been attached to many different biological compounds such as DNA, other proteins, or fluorescent tags. With this technology we can pair a Cas9 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 donor 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 specific combos of RNA and donor DNA that enabled selection of multiplexed edits within a population of cells.

In the end, we have created a Lego-like tool where a variety of different components can be attached to genome editing machinery 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.