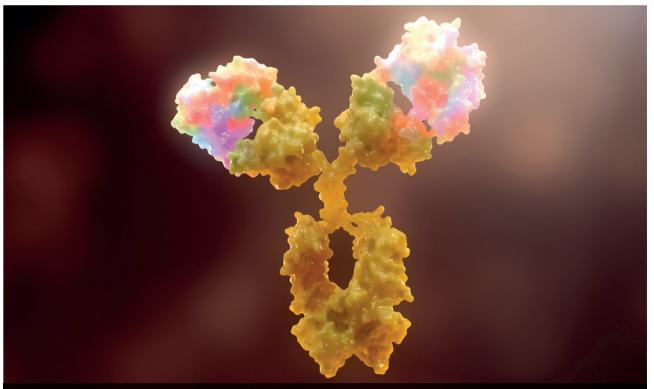
How to make random mutagenesis less random

By merging large-scale, controlled DNA SYNTHESIS WITH SEMICONDUCTOR TECHNOLOGY, antibody engineering via directed evolution is becoming quicker, easier, and more rational.



GENSCRIPT

Antibodies can be engineered to improve many of their qualities, but directed evolution through random mutagenesis can introduce unintended bias.

Atypical hemolytic uremic syndrome is an ultra-rare genetic disease that causes anemia and damages kidney function. There is an approved treatment — a therapeutic antibody called eculizumab — but its half-life of only 11.3 days means patients need to endure transfusion every two weeks.

In 2018, researchers at Alexion Pharmaceuticals, maker of eculizumab, described their efforts to extend its half-life and lessen the infusion burden for patients. Years of working with the antibody had given them a deep knowledge of its structure. The researchers used a rational design strategy to mutate specific amino acid residues. Four substitutions were identified that resulted in a doubling of the eculizumab half-life when tested in a mouse model[1].

But what if there had been no background information? Or what if a greater number of substitutions, or combinations of substitutions, needed to be tested? The number of possible changes, even within a short region of an antibody, can rapidly become unwieldy, making rational design using site-directed mutagenesis impractical. This is where directed evolution strategies come in.

Random mutagenesis

Directed evolution experiments start with large libraries of mutants created through random mutagenesis, typically involving error-prone PCR or mutagenic chemicals. One of the most common PCR-based techniques is saturation mutagenesis, which uses oligonucleotides with degenerate codons to produce all possible amino acid combinations at specific positions in a protein.

However, that 'random' part of the mutagenesis is also where trouble can start. In 2015, a chemical-statistical analysis of experimental saturation mutagenesis datasets found that factors such as DNA sequence, G/C content, randomization scheme, and primer quality, can all influence, and even bias, the final composition of a saturation mutagenesis library.[2]

This possibility of mutation bias and the introduction of stop codons means researchers must screen clones from the library following saturation mutagenesis, in order to determine which mutations are present and at what frequency, adding significant time and costs. In an attempt to improve this, developers have been working on ways to bridge the rational design/directed evolution divide.

Rationalizing directed evolution

Biotechnology firm, GenScript, in Piscataway, NJ, is one such developer. GenScript's strategy for saturation mutagenesis

FINDING OPTIMIZED ANTIBODIES WILL BE QUICKER AND MORE EFFICIENT

relies on programmable semiconductor chips, which are DNA synthesis platforms with high oligonucleotide synthesis capacity, combined with user-defined control over every oligonucleotide sequence.

Each semiconductor chip contains thousands of arrays of platinum electrodes that can each synthesize a unique oligonucleotide. Currently, one semiconductor chip can produce between 12,000 and 92,000 oligonucleotides, ranging in size from 10 to 170 nucleotides. However, it's the system's ability to independently control exactly what is being synthesized at each electrode that sets this approach apart from traditional random saturation mutagenesis.

By using the programmable semiconductor platform, it becomes possible to design and plan a saturation mutagenesis experiment, specifying both the desired mutations and the frequency that these codon changes will appear in the library. A researcher using this approach can ensure the desired balance of substitutions and protein variants in their mutant library to identify the optimal protein candidate, without the need for additional screening or mutagenesis[2].

Finding that antibody in a haystack

Continuing to advance antibody engineering strategies is critical to the discovery of new therapeutics, and, as seen with eculizumab, for enhancing the characteristics of existing antibodies. As technology allows directed evolution approaches to become more like rational design, finding optimized antibodies will be quicker and more efficient, paving the way for more therapeutic antibodies to enter the clinic.

1. Sheridan. D. et al. PLoS One 13(4): e0195909 (2018).
2. Acevedo-Rocha, C.G., Reetz, M.T. & Nov, Y. Sci. Rep. 5:10654 (2015).

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