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OC002 Saturation mutagenesis and precision libraries: the numbers game

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Saturation mutagenesis is a vital tool in the protein engineer's arsenal, but involves astronomical numbers of genes when targeting multiple codons. Some argue that an iterative codon-by-codon approach is the answer, whilst others are convinced that owing to context-dependency, iteration will exclude fundamental best "hits". Meanwhile, some advocate creating vast "sampling" libraries while others champion full representation. Whatever the stance, the numbers game in saturation mutagenesis plays a vital role in the eventual outcome of a protein engineering project.

Embracing minimalist, non-degenerate saturation involves extra work up-front, but minimizes the numbers effect during the most intensive part of the whole process – namely screening the resulting libraries. Appropriate non-degenerate saturation can eliminate undesirable residues prone to oxidation or helix disruption or can reduce the frequency of undesirable dipeptide occurrences, while much smaller ligand-specific libraries can be created exclusively. Different applications such as antibody loops or active sites of enzymes require different synthetic protocols to generate non-degenerate saturation libraries. This presentation will examine the numbers, the various platform technologies

created in our lab over the last decade, whether proprietary or open access, consecutive or disperse codons, manual or automated synthesis and the quality of resulting hits that require no further affinity maturation.