Follow us:

Abstract Details

Title: **MAX randomisation: designed, non-degenerate saturation mutagenesis of armadillo repeat proteins.**

Authors: Anupama Chembath - *Aston University, United Kingdom*; Mohammed Ashraf - *Aston University*; Ben Wagstaffe - *Aston University*; Yvonne Stark - *University of Zurich*; Andreas Plückthun - *University of Zurich*; Anna Hine - *Aston University*

Session: Poster Day 2, , 00:00 - 00:00

Non-degenerate saturation mutagenesis is critical to library composition both in terms of library size and amino acid representation. Unlike conventional methodologies, non-degeneracy permits equal representation of all encoded amino acids and effectively eliminates termination codons from saturated positions. We have previously described both 'MAX' randomisation, which saturates physically separated codons and 'ProxiMAX' randomisation, which saturates contiguous codons. Both allow the additional advantage of encoding only required amino acids without reference to codon sequence, but their methodologies are quite different. Whilst MAX randomisation employs a manual process of selectional hybridisation between individual oligonucleotides and a conventionally-randomised template, ProxiMAX relies on saturation cycling; repeated cycles of blunt-ended ligation, Type IIS restriction and PCR amplification. MAX randomisation is thus typically employed in the research laboratory to engineer active sites of enzymes (or α-helices within other proteins), whilst ProxiMAX is now a chiefly commercial, automated process employed in antibody engineering. Here we present the application of MAX randomisation to engineer libraries of Armadillo Repeat Proteins (ArmRPs), α-helical proteins that selectively bind extended peptides. We have utilised the MAX randomisation technique to engineer ArmRPs for the generation of gene libraries encoding multiple repeat modules, saturating seven positions and encoding between 4 and 18 amino acids within each location, achieving excellent correlation between the design and observed specifications. We also present early developments to extend MAX randomisation into the realm of multiple contiguous codons.


powered by
eventsforce