**The Hopeful Cornucopia – a Study into Combinatorial Gene Synthesis**

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Diverse libraries of structural genes offer an attractive option of prospecting for proteins with new catalytic properties. At a given library diversity and a given function to be searched for, the odds of finding a carrier are largely governed by the extent to which the ensemble is populated by molecular species with amino acid exchanges located at positions relevant for substrate binding and catalysis. This defines two pre-requisites: *(i)* The availability of a protein scaffold that allows conceptual separation of these "relevant" positions from the others and *(ii)* a method of gene synthesis that allows addressing diversification to these positions exclusively.

We have implemented these pre-requisites in the following way: *(i)* A thermostable TIM-barrel protein (tHisF[1]) was chosen as the starting scaffold. *(ii)* A hierarchically structured, modular regime of gene synthesis was applied in which codon positions to be randomized are served by mixtures of trinucleotide coupling blocks.

Specifically, in two rounds of gene library construction, 26 (24) residues were addressed by trinucleotide mixtures which had been prepared in such a way as to yield, on average, 7.3 (6.7) amino acid substitutions per molecule (binomially distributed) – with a substitution bias in favour of amino acids preferentially exposed on protein surfaces and/or present in active sites of enzymes.

Resulting libraries were analyzed in three stages: *(i)* Several hundred clones were picked at random and subjected to DNA sequencing in order to assess the quality of the synthetic procedure. *(ii)* A subset of clones was inspected for folding stability of the encoded proteins in order to assess the validity of the starting assumptions about participation of individual residues in substrate binding and catalysis or in the provision of folding stability. *(iii)* Searches – by genetic complementation – of the libraries for molecular species meeting certain pre-set functional requirements have been initiated and have yielded first results.

[1]Sterner, R. and Höcker, B. (2005) Catalytic Versatility, Stability and Evolution of the $(\beta\alpha)_8$-Barrel Enzyme Fold. *Chem. Rev*. **105**, 4038 – 4055.