Chapter 10: Summary
In recent years, the concept of *de novo* design and laboratory evolution has gained great importance especially as an invaluable tool in designing novel bioactive molecules. Here, an application of the 'codon-shuffling' method, a technique for laboratory evolution of enzymes/proteins is described towards the creation of *de novo* protein libraries of use. From the initial idea of using the DC set for making *de novo* proteins, the method for library creation has been streamlined, by designing tailored hairpin scaffolds that encapsulate the DC proteins favorably. The method described in this report results in *de novo* proteins and not just peptides, because of which the library would be much less amenable to proteolysis by cellular proteases. The longer average length of the library members also presumably enhances protein folding. Secondly, an exclusive property of the 'codon-shuffling' method has also been explored, which is the allowance for skewing library properties by inclusion, exclusion or predominance of some DCs. The ability to skew a protein library would further narrows the protein space that needs to be explored. One can therefore design skewed libraries to isolate proteins for specific needs. For example, a protein library of arginine-rich sequences may be generated by an overwhelming use of the ‘RT’ dicodon; poly-arginine peptides have lately gained much interest for their ability to act as cell-penetrating peptides that act by binding to the cell-surface of a pathogen (Goncalves *et al.*, 2005). Furthermore, the recent sequencing of organisms such as *M. tuberculosis* and *P. falciparum* has unearthed many virulence-determining proteins that are exclusively composed of short sequence repeats (Ravi Chandra *et al.*, 2005). It is not known what selective advantage this may provide the pathogen. Proteins rich in DC-repeats, constructed using our method could serve as a model for studying this hitherto unexplored natural phenomena. Finally, our proof-of-concept experiments relating to the generation of AMPs against *E. coli* can be conveniently extended to pathogenic microorganisms like *M. tuberculosis*. The molecular targets of AMPs can then be identified using DNA microarrays. Such studies, would provide a novel direction towards discovering new molecules against common pathogens, and importantly, would further extend the scope of *de novo* protein design as an important chemical tool for tackling generic problems in medicine.

The work done can be summarized as follows:

1. The codon shuffling method has been demonstrated and streamlined towards creation of helical hairpin encapsulated protein libraries that
can generate proteins of sizes varying from ~2kDa to 15 kDa. It has been shown that the ‘codon-shuffling’ method can be employed both as a non-rational and part-rational approach as the case may demand.

2. Unlike degenerate libraries based on oligonucleotides, selection in codon shuffling is not limited by length.

3. Using ‘codon-shuffling’ as a method of de novo design, now proteins can be generated and not just peptides. Therefore, here is greater chance of having proteins with some secondary structures and folds, given the natural preference for DC proteins as described earlier.

4. Proteins are less amenable to in vivo proteolytic degradation than a peptide.

5. An exclusive advantage of DC-method over any other existing method has been unleashed for generating peptide library that is, skewing the amino acid content of the DC-protein. For example, to design a more basic protein, ‘KL’ and ‘RT’ content of DC’s can be increased. Similarly, for a more acidic protein, ‘DI’ and ‘EL’ content can be increased.

6. Using the ‘codon-shuffling’ method, equimolar as well skewed hairpin encapsulated protein libraries has been created towards selection of antibacterials.

7. From the equimolar library, three antibacterial proteins, EQAMP1, EQAMP2 & EQAMP3 have been selected that were found to be bacteriostatic as monitored by growth inhibition in liquid/plate culture as well as by TEM analysis.

8. From the KL, RT skewed library, one antibacterial protein, SKAMP1 has been selected that was found to be bacteriostatic as monitored by growth inhibition in liquid/plate culture as well as by TEM analysis.
References


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