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Mini Review

Protein Engineering; Structural Manipulations Leading to Desired Properties. A Mini Review

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Abstract

Proteins; the workhorses of the cells of living organisms, are produced from instructions encoded into cellular DNA. The protein identity is dictated by a unique sequence of twenty chemicals known as amino acids which first come together in various combinations to form longer chains of polypeptides. So, called because they are held together by covalent linkages-the peptide bonds, these polypeptides in turn assume a 3D structure augmented by various non-covalent associations such as hydrogen bonding, electrostatic interactions, hydrophobic forces and van der waals forces and often the covalent disulfide bonds. This allows proteins the liberty of being structural and functional building blocks in the cells, earning the reputation as "workhorses of the cells" and playing many roles in cellular physiology [1].

Keywords: Protein Engineering; Proteins

While exhaustive literature is available on the nature of proteins and their function, protein engineering is relatively a new dimension in biochemistry graced particularly after the surge in developments of molecular biology. Protein engineering, which first began in the early 1980s, comprises the use of genetic or chemical techniques to study and modify the protein's structure, to increase the stability of protein, or to vary its physiological function or activity [2]. Protein engineering thus becomes the art of altering the properties of a protein by making deliberate changes in its primary structure. It allows the introduction of predesigned changes into the gene for synthesis of a protein with an improved function that is desired for its application [3]. The technique involves two different strategies of random-mutagenesis and site-directed mutagenesis (SDM). The goal is to increase the effectiveness of proteins for commercial or medical applications, and to modify the substrate specificity of enzymes or enhance their catalytic efficiency [4].

During the previous two and half decades, a breakthrough in molecular biology and biotechnology has opened the door to an entirely new discipline focused on nano-scale engineering of highly functional biomolecules. The field of protein engineering has evolved rapidly since then, and only in the last 15 years has technically advanced approaches to protein engineering developed in solving a wide variety of practical, real-world problems.

Site specific mutagenesis

One of the most important advances in biology includes the development of site-specific mutagenesis of DNA, some thirty years ago, which is done by the site-specific replacement of any amino acid in a protein with one of the other nineteen amino acids. This led to the new era of protein engineering and opened doors to its applications in different fields of life [5].

An interesting case arises with the application of protein engineering in Alzheimer's treatment. Alzheimer's disease, the most common form of dementia, having no cure at present, is caused by damage to the nerves in the brain. This damage is caused by oligomers of the amyloid ß peptide (Aß), a kind of precipitate that accumulates in the brain [6]. For the first time, using protein engineering, a protein is being constructed by researchers named affibody protein, which has yielded experimental results that are promising when it comes to stopping the disease. This artificial so-called affibody protein prevents the formation of toxic forms by entirely enclosing the Aß peptide. The current approach is to alter the characteristics of the affibody proteins, which are the small proteins being engineered to bind to a large number of target proteins or peptides with high affinity, imitating monoclonal antibodies, so it will not be broken down when it enters the blood [7]. The strategy is also being tested in flies, and the initial results recommended that the strategy works.

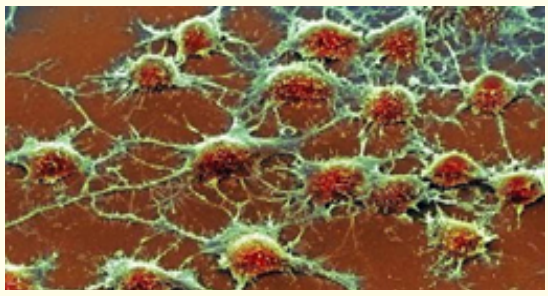

Figure 1: Alzheimer's disease culture cells. These cells have been genetically engineered to produce amyloid precursor protein (APP). (Figure is adapted from Simon Fraser).

Yet another strategy involves stabilizing the toxic oligomers that are the cause of nerve cell death and memory loss. Determining the 3D structure of the oligomer would be very helpful in this regard, and it is important to further study the molecular mechanisms which may lead on to drug development in this direction.

Revolution of protein therapeutics by customization of existing proteins or creation of novel proteins for specific clinical applications is one of the major areas of protein engineering applications. An increasing number of engineered protein therapeutics are currently being developed, tested in clinical trials and marketed for use. Here, a brief history and futuristic approach to protein therapeutics is being discussed. A variety of stratagems have emerged for modulating protein properties, such as efficacy, stability, specificity, immunogenicity and pharmacokinetics (PK). Several mechanisms are present for altering these properties including manipulation of primary structure by replacing a protein that is

deficient or abnormal, or augmenting an existing pathway, incorporation of chemical and post-translation modifications to provide a novel function or activity such as a radionuclide, cytotoxic drug, or protein effector, and utilization of fusion partners like Fc fusion proteins.

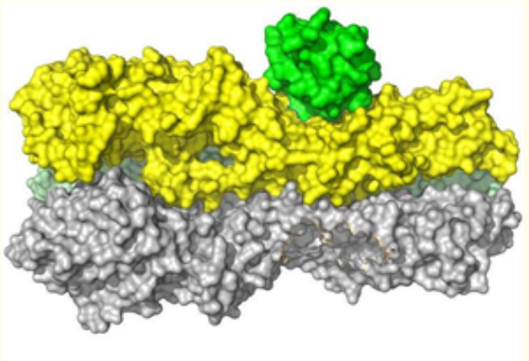

Figure 2: Engineering a new way to block the flu virus. A bright-green engineered protein molecule is bound to a portion of the influenza virus (in yellow and gray), showing the complicated surface with its crevices and bulges (Figure is adapted from Keith Seinfeld, 2011).

Over the past years, protein therapeutics has attained a drastic evolution with protein engineering tools, starting from insulin as the first recombinant human protein therapeutic, followed by the advent of fusion proteins engineered by uniting the genes encoding two or more different proteins. Romiplostim approved as a human therapeutic for treatment for thrombocytopenia is one of the latest example.

Other engineering protocols to increase the clinical potential of proteins involves the conjugation of proteins to chemicals such as radionuclide chelators, cytotoxic drugs and PEG which can grant proteins with brand new capabilities. Amongst all, PEG is the most clinically and commercially successful and by far at least 8 PEGylated proteins are approved as human therapeutics [8]. Furthermore, the development of the "engineered protein scaffolds" adds another interesting aspect having potential advantages over antibodies. These are conceived to include smaller size and higher stability over antibodies and more efficient tissue penetration. Such successful clinical and commercial experience with protein therapeutics has provided an understanding of their strengths and limitations and motivation to develop better protein drugs.

The synthetic protein certifies a new approach that allows designing functional artificial proteins using algorithms. Advanced tools are used to engineer more effective antibodies and other significantly beneficial proteins, with shapes that don't exist in nature [9]. Re-engineering a natural protein for a new purpose is a serious challenge due to amino acid interdependency and multiple-utility of amino acid, which was also faced in creation of the first completely Artificial Oxygen Transport Protein [10]. This approach also agrees to reconsider the design of natural oxygen transport globin. Similarly, "*in silico*" re-engineering is attracting many researchers working in this area of biochemistry. Scientists use sophisticated protein modeling software like ROSETTA and DEZYMER, which includes the related laws of physics and chemistry to find amino acid sequences that fold into stable forms and have specific functions [11].

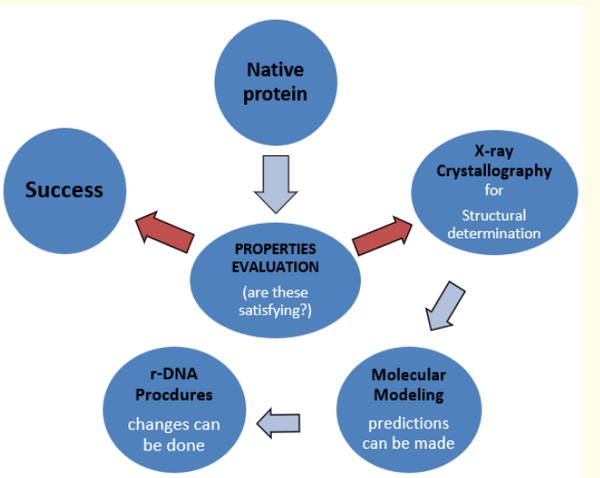

Figure 3: Strategies for Rationale Designing.

Genetic engineering of protein toxins

Fusion or hybridization of protein toxins are used in advanced therapeutics. Most of the protein toxins comprised of two or more Many protein toxins consist of two or more functionally diversified domains like binding domain specific for binding of target cell, antigen or a receptor while a catalytic domain interrupt metabolic processes migrates across the membrane [12]. Therapeutic fusions are created by the scientists from the protein toxins of ricin, diphtheria toxin or Pseudomonas exotoxin by splicing the reduced catalytic domains onto the growth factors or antibodies that selectively binds to proteins present on cell surface that are associated with various autoimmune diseases and cancers [13]. The advantage of this strategy is that these antibodies spare healthy cells thus, only recognize and kill diseased cells thereby, are being evaluated for cancer immunotherapy. One such genetically engineered immunotoxin has already been commercially marketed for the cutaneous T-cell lymphoma treatment [14].

Yet another significance of fusion protein toxins includes vaccines that transport bacterial antigens to leucocytes [15]. Fusion toxins are often characteristically different from their ancestor toxins. For example, catalytic domains of tetanus and anthrax toxin are coupled, and the resultant hybrid is capable of entering and killing non-neuronal cells, and thus is more potent than any of the parent [16]. Moreover, the fusion toxin strategy can improve the binding efficacy and affinity of protein in killing the target cells; in one such experiment, these techniques were used to increase the cytotoxicity of a fusion protein toxin, up to 17-folds [17].

Conclusion

Proteins are an attractive platform, because they can be more easily 'engineered' as opposed to other biomolecules and being utterly ubiquitous and important in cellular physiology impact many chemical reactions. For the future, protein engineering methods based on separation of desirable from undesirable changes, and their amalgamation into the protein being engineered will become ever more powerful, as sequence information continues to increase exponentially. The ability to create novel biomolecular

structures, with enhanced functional properties, will be a powerful means of addressing key technical challenges concerning the diversity of this technique.

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