LECTURE-18

PROTEIN ENGINEERING

A. DEFINITION

- Modification of protein structure with recombinant technology or chemical treatment to get a desirable function for better use in medicine, industry and agriculture.
- It enables scientists to create unique materials that do not occur in nature.

B. APPROACHES OF PROTEIN ENGINEERING

Protein engineering consists of three major approaches:

1) Rational design involves knowledge based mutagenesis (KBM)

- Scientists use detailed knowledge of the structure and function of the protein to make desired changes.

  **Advantages:**
  - Technically easy: Allows scientists to change structure of a protein in a predictable way.
  - Relatively inexpensive

  **Disadvantages:**
  - Detailed structural knowledge of the protein is often unavailable.
  - It is extremely difficult to predict the effects of various mutations.

2) Computational protein design (CPD) or De Novo approach

- Proteins are computationally designed from the level of amino acids to the level of a functional protein complex.
- This approach uses molecular modelling programs to predict amino acid sequences that will fold into a desired structure.
- Design scheme may encompass small regions of the proteins or the entire protein.
- Design may aim at the side chains or at the full backbone confirmation.

  **Advantages:**
  - It has unique ability to design function du novo.
  - It creates proteins with functions that are not available in naturally occurring proteins.
  - Allows designing proteins with no human intervention in sequence selection.

  **Disadvantages:**
  - Tedium and complex process.
3) **Directed evolution (DE)**
- Mimics the process of natural selection to steer (guide) proteins or nucleic acids towards a user-defined goal.
- Introduce desired properties into proteins via random mutation or gene recombination.
- **Advantages:**
  - Can be performed without knowing every detail of a protein’s structure.
  - Library size limitation can be overcome by creating libraries of variants processing desired properties.
- **Disadvantages:**
  - Time consuming and expensive process.

![Protein engineering diagram](image)

*Figure 1: Different approaches of protein engineering*
### C. METHODS OF PROTEIN ENGINEERING

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| 1.     | Localized or region specific random mutagenesis | • Combination of rational and random approach  
• Includes simultaneous replacement of a few amino acid residues in a specific region                                                                                                               |
| 2.     | DNA shuffling                              | • Group of genes with a double stranded DNA and similar sequences is obtained from various organisms.  
• Genes are digested and yields randomly cleaved small fragments (DNaseI).  
• These fragments are purified and reassembled by PCR (DNA polymerase).  
• Hybrid DNA with parts from different parent gene is obtained.                                                                                                                  |
| 3.     | Peptidomimetics                            | • Includes a variety of synthesis methods such as use of a common intermediate, solid phase synthesis etc.  
• Involves mimicking or blocking the activity of enzymes or natural peptides upon design and synthesis of peptide analogs that are metabolically stable.                                    |
| 4.     | Staggered extension process                | • It is the variation of DNA shuffling method.  
• Involves the use of a mixture of restriction endonuclease instead of DNaseI.  
• It does not require parental gene fragmentation.                                                                                                                                     |
| 5.     | Flow cytometry                             | • Powerful method for single cell analysis.  
• Ex: Enzyme engineering of intra or extracellular enzymes.                                                                                                                                |
| 6.     | Cell free translational system             | • Alternative to in vivo protein expression.  
• When template DNA or RNA is added to a reaction mixture, proteins are produced upon incubation in the absence of cells.                                                                 |
| 7.     | Stimulus responsive enzyme system          | • Based on both naturally existing peptides and rationally engineered systems.  
• Based on the fact that peptides and proteins are able to change their confirmation as a response to external stimulants such as temperature, pH or some specific molecules. |
8. **Designed divergent evolution**
- Used in redesigning enzyme functions.
- Based on the theories of divergent molecular evolution.
- Firstly enzymes with more specialized functions have evolved from those enzymes with promiscuous functions.
- Secondly, the process is driven by a few amino acid substitutions.
- Finally the effects of double/multiple mutations are usually additive.

9. **Receptor based QSAR methods**
- Based on a computational combination of structure activity relationship analysis and receptor structure based design.

10. **Phage display technology**
- Relates phenotypes with their corresponding genotypes.
- Particularly used in “synthetic binding protein engineering”.

11. **Yeast surface display**
- Many different proteins can be displayed on yeast surface, and the yeast secretory biosynthetic system promotes efficient N-linked glycosylation and oxidative protein folding.

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### D. APPLICATIONS OF PROTEIN ENGINEERING

1) **Industrial Applications**

- **Food Industry:**
  - Proteases: Production of low allergic infant formulas, milk clotting and flavours.
  - Amylases: For liquefaction and saccharification of starch, in adjustment of flour and bread softness
  - Lipases: For stability and conditioning of dough

- **Detergent Industry:**
  - Proteases: For removing protein stains
  - Amylases: In removal of starch stains
  - Lipases: In removal of lipid stains

2) **Environmental Applications**

- Detoxification of inorganic pollutants (phenols, azo dyes, organophosphorous pesticides etc.) using enzymatic oxidation.
- In petrol biorefining: Denitrogenation of fuels, heavy metal removal, fuel biodesulfurization
• For improvement of hydrogen peroxide stability, increasing the redox potential to broaden the substrate range.

![Diagram](image)

**Figure 2: Various applications of protein engineering**

3) **Medical Applications**
   - In pretargeted radio immunotherapy
   - To improve pharmacokinetic properties of antibodies
   - To modify antibodies to target cancer cells for clinical applications

4) **Biopolymer Production**
   - To produce peptide based biomaterials, such as silk like polymers
   - To create and improve protein domains, hereby producing new biomaterials.

5) **Nanobiotechnology**
   - Phage display and bacterial cell surface display methods are used to select polypeptide sequences which selectively bind to inorganic compound surfaces, for applications of Nanobiotechnology
   - For nanowire construction
6) Redox Proteins and Enzymes

- Such proteins and enzymes can be modified to be used in nano devices for biosensing.
- Two emerging areas of such proteins and enzymes are: nucleic acid based catalyst construction and intra molecular electron transfer network remodelling.

7) Other new applications

- Insertional protein engineering
- Zinc finger protein engineering
- Virus engineering
- Protein casein modifications

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