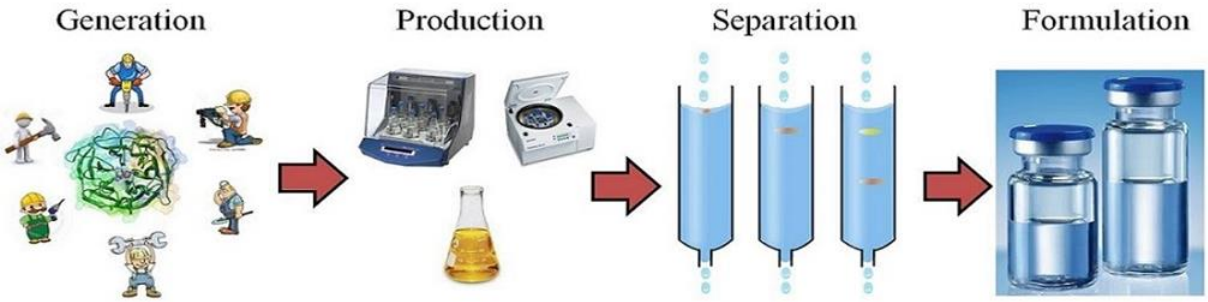
Protein Pharmaceutical Lab

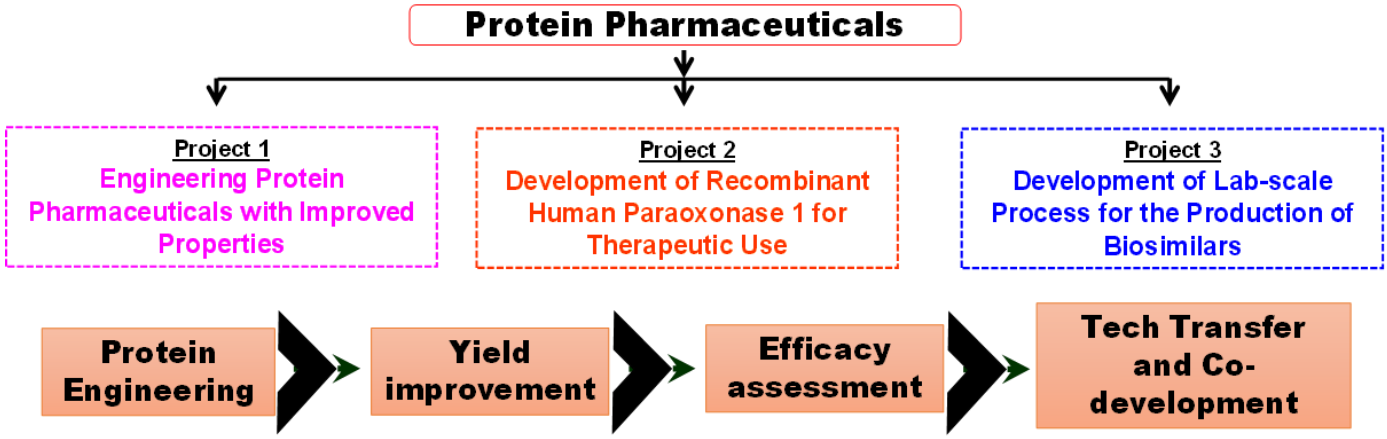


The laboratory is involved in the development of Protein Pharmaceuticals and is situated in the Department of Biotechnology at the National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab, India.

The laboratory is led by **Dr. Abhay H. Pande**, Associate Professor, Department of Biotechnology, NIPER, S. A. S. Nagar. Dr. Pande has >22 years of experience in the field of protein research. He obtained his M.Sc. degree in Biochemistry in 1995 from Nagpur University, Nagpur and Ph.D. degree in Life Sciences in 2000 from D.A.V.V. Indore. Subsequently, he obtained nearly five years of post-doctoral research experience by working in national (Dr. Girish Sahni, IMTECH, Chandigarh) and international (Dr. Suren Tatulian, University of Central Florida, USA) laboratories. Prior to joining NIPER in 2006 he also held an academic at the Department of Biological Sciences, Birla Institute of Technology and Science, Pilani.

CURRENT RESEARCH

Protein pharmaceuticals are mostly recombinantly-produced proteins that are used for the therapeutic purpose. Over the past decades, advance in the development of technologies has brought hundreds of therapeutic proteins into the clinical use. It is evident now that, in coming decade, the domestic as well as the international market for protein pharmaceuticals will grow rapidly and will expand its share of the entire pharmaceuticals market.



Project 1: Engineering Protein Pharmaceuticals with Improved Properties:

Low *in vivo* efficacy of protein pharmaceuticals is also attributed to their poor pharmacokinetics (because of their low circulatory half-life). This is **a major problem** in the clinical use of these proteins and engineering recombinant proteins having increased circulatory half-life **is the need of the hour**. In this project, we are trying to develop novel, long-acting variants of recombinant human arginase 1 and endostatin for cancer treatment.

Long-acting Arginase 1: Several tumors are auxotrophic to arginine and deprivation of arginine leads to tumor reduction. Administration of recombinant human arginase 1 have been shown to reduce cancer and thus has emerged as a promising therapeutic candidate against several cancer. However, the protein exhibits low circulatory half-life (~4.5 h). To address this, we have engineered and expressed long-acting variants of human arginase 1 (**Fig. 1A-B**).

Long-acting Endostatin: Endostatin, a 20-kDa fragment of type XVIII collagen, is clinically used as an anti-angiogenic agent (broad-spectrum inhibitor of angiogenesis). However, recombinant human endostatin exhibits poor *in vivo*

pharmacokinetics. In our laboratory, we have designed and expressed long-acting variants of human endostatin (**Fig. 1C**).

Currently, we are in process of characterization of these novel proteins.

Project 2: Development of Recombinant Human Paraoxonase 1 for Therapeutic Use:

Current treatments available for Organophosphate (OP)-poisoning are inadequate and unsatisfactory and more effective treatment is urgently needed. Human paraoxonase 1 (h-PON1) can inactivate nerve agents/pesticides and is a new generation antidote for the pre-treatment of OP-poisoning in human. H-PON1 also exhibit anti-inflammatory, anti-oxidative, anti-atherogenic, and anti-diabetic properties and administration of recombinant PON1 have been shown to prevent/retard the development of various diseases (e.g, coloitis, stroke, hyperlipidemia, atherosclerosis, diabetes) in animal models. Thus, h-PON1 is a strong candidate for the treatment of various disease in human (either alone or in combination with existing therapies). However, there are numerous limitations regarding large-scale production and use of h-PON1 as a therapeutic candidate which include low enzymatic activities of native h-PON1, difficulties in expression & purification of recombinant h-PON1, and poor stability of purified enzymes.

In this project we are trying to address these issues. By using random and rational mutagenesis approaches, we have generated variants of rhPON1 having increased activity. A simple and cost effective method for mass production of rh-PON1 enzymes is also developed (**Fig. 2A**). To increase the *in vivo* pharmacokinetic properties, long-acting variants of this enzyme are also designed (**Fig. 2B**). We are in process of characterization of these novel proteins.

Project 3: Development of Lab-scale Process for the Production of Biosimilars:

Biosimilars are recombinantly-produced protein molecules that are very similar to their 'native' counterparts in term of their biological effect(s). The main goal of this project is **to develop lab-scale technologies for the coast-effective production of biosimilar using *E. coli* expression system**. Towards this, we have cloned and expressed a variety of biosimilar molecules (viz., human enzymes, interferons, growth factors and hormones) (**Fig. 3**).

Papers

51] DharamPal*, Patel, G.*, Dobariya, P., Banerjee, U.C. and **Pande, A.H.** Optimization of growth medium for cultivation of recombinant escherichia coli se1 expressing human interferon-β using response surface methodology. (Submitted)

50] DharamPal*, Tripathy, R.K.*, Teja, M.S. Kumar, M., Banerjee, U.C. and **Pande, A.H**. Antibiotic-free expression system for the production of human interferon-beta protein. (Submitted)

49] Agarwal, G., Tripathy, R.K. and **Pande, A.H.** Production of recombinant human liver prolidase by in vitro refolding.

48] Tripathy, R.K., Aggarwal, G., Bajaj, P., Kathuria, D., Bharatam, P.V. and **Pande, A.H**. Towards understanding the catalytic mechanism of human paraoxonase 1: mutagenesis and in silico studies. Appl. Biochem. Biotechnol. 182, 1642-1662.

47] Iyengar, Satvik A.R. and **Pande, A.H.** Organophosphate-hydrolyzing enzymes as first-line of defence against nerve agent-poisoning: Perspectives and the road ahead. Protein J. 35, 424-439

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Patents:

1. **Abhay H. Pande**, Priyanka Bajaj, Rajan K. Tripathy, Geetika Agrawal. Recombinant human paraoxonase 1 enzymes, method of generation and uses thereof. (Patent pending, application # 228/DEL/2013 & PCT/IB2014/058461).

2. **Abhay H Pande**, Sunil A. Nankar. Anti-inflammatory peptides. (Patent pending, application # 713/DEL/2013)

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