Team of Bio-macromolecule Process Engineering
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**Brief Introduction**

The Team focuses on basic principles of large-scale cell culture, efficient product separation/purification, molecular modification and applications of bio-macromolecules including protein pharmaceuticals and vaccine through multi-level and multi-scale analysis.

The aim is to develop highly efficient bioprocesses for vaccine, antibody, cytokine, peptide, and enzyme production. The following technical and scientific principles have been addressed: efficient expression of proteins in mammalian cells, yeasts and bacteria, bioreactor design and mass transfer, cell disruption and separation, inclusion body processing, membrane filtration, chromatography purification, and site-specific modification. The denaturation mechanism of the target products, including the effects of microenvironments, the solid-liquid interface, and the operating conditions is particularly emphasized. By combination of theoretical analysis and practical approach, anti-denaturation strategies for complex bio-macromolecules such as conjugated proteins, virus-like particles (VLPs), and enzyme are developed. The team has been cooperating with industrial companies to apply novel technologies in real production process for increased quality, safety, compatibility and reproducibility. New processes have been developed for several new products that have gained commercial successes.

**Research Activities**

1. **From laboratory to industrialization of purification technology for a novel Hepatitis B vaccine**

A series of innovative technologies for bioactivity protection of vaccines during chromatographic separation and purification were designed, which included application of new chromatographic media, integration of chromatography system suitable for rapid purification, as well as chaperon-assisted purification process to prevent the large vaccine antigens from denaturation or deactivation. Such strategies have been applied in industrialization of recombinant HBsAg derived from yeast Hansenula. The process was built in Hualan Biological Engineering Inc. of China. A production line for five million dose vaccination was set up. Compared with former technology, the recovery was increased from less than 20% to more than 35%. This process showed good batch-to-batch repeatability and had been used for more than 3 years.

2. **Designing and manufacturing of instruments for purification and analysis of biotechnological drugs**

The objective of this project is to design and develop a high-standard chromatography workstation for separation and purification of biotechnology pharmaceuticals. The design was based on innovation patent which allows for fast, accurate and economical characterization of the biomolecules. The main content of this project concerns with the developing of new precision flow-distribution technology for multi-channel detection, novel chromatographic media for protein separation, uniform flow distributor for column geometry etc. This instrument will be used for both analysis and preparation of recombinant pharmaceutical protein, long-acting protein drugs, vaccines and blood products, etc.
3. Biotech drug candidates study

The task of this project is to evaluate the efficacy of selected antibodies, vaccines, and peptides for the treatment of neurodegeneration, cancer, type 2 diabetes mellitus. The mechanism and the safety of these drug candidates are investigated, including in vitro and in vivo experiments. The objective is to develop novel pharmaceuticals with improved efficacy, better circulation, site-targeting, and new applications.

4. Industrial bioprocess intensification and integration

This project is aimed at to investigate the basic principles governing the efficiency of mass transfer, energy transfer and fluid flux in bioreaction and bioseparation process, to develop new de-bottlenecking technologies and methods on the basis of theoretical analysis and mathematical modelling on the complex bio-systems, and finally realize bioprocess intensification and systems integration. Basic principles governing structure and activity conversion of enzyme molecule in complex microenvironment, cellular substance synthesis and transport, fluid flux in extracellular restrict space, and basic mass energy transport law in bioreactors will be investigated and emphasized.

Infrastructure

The team is equipped with various instruments to provide solid support for both basic study and scale-up production biomacromolecule including proteins and vaccines. The instruments can be generally classified into the following four categories:

1. Cell culture platform for both microorganism fermentation and mammalian cell line to produce protein, antibodies and vaccine with scale from 5 L to 5 t.;
2. Biomacromolecule separation/purification platform including pilot-scale fast preparative liquid chromatography and membrane concentration instrument;
3. Analytical platform for macromolecule characterization, identification, structure and stability analysis, which include HPLC, Isothermal titration calorimetry, MicroCal VP-DSC microcalorimeter, and multi-angle laser light scattering detector, Circular dichroism spectrophotometer, Malvern ZetasizerNano ZS, et al.,
4. Self-constructed multidimensional integrated protein chromatography workstation, which can work sequentially, in parallel or in a mixed mode to enable simultaneous separation and purification of various proteins from a complex mixture.

Meanwhile, the group also established several of joint laboratories with innovative companies, which include GE Healthcare, Hualan Biological Engineering Inc., et al. Based on these Joint labs, more than 1000 biochemical engineers from all over the country participated in the short-term training organized by SKLBE during the past five years.
The Group has 21 stuffs, including 7 professors, 4 associate professors, 8 assistant professors, 2 administrators, and 25 graduate students.

**Research Progress**

By combination of theoretical analysis and practical approach, the team developed different anti-denaturation strategies for complex biomolecules such as conjugated proteins, virus-like particles, and enzymes.

1. **Low-density flexible ligands for adsorption and separation of bio-macromolecules**

During chromatographic process, possible strong interactions between chromatographic media and multi-subunit viral or VLPs vaccines is one of the most important reason causing significant aggregation or dissociation of VLPs. To reduce such intensive adsorption-desorption during chromatographic process, lowering ligands density of the chromatographic media was found to provide an effective remedy to the low activity recovery of viral or VLPs antigens. One example is the hydrophobic interaction chromatography (HIC) process of Hans-rHBsAg. Through regulation of ligand density, the surface hydrophobicity of the adsorbent was deliberately manipulated, resulting in an extraordinarily improved purity and recovery of CHO-rHBsAg during the HIC process. Similar results was observed in the ion-exchange chromatography (IEC) of rHBsAg, whose recovery could be increased from about 44% to 66% and the purification fold was increased from 9 to 13 by decreasing the ligand density from 0.13 to 0.04 mmol DEAE/ml adsorbents.

2. **Gigaporous chromatography of VLPs**

VLPs are self-assembled from unusually more than 100 subunits and have size ranging from tens up to hundreds of nanometers. Unfortunately, common chromatographic packing beads have pore sizes in the range of 20-30 nm which close to the size of VLPs and may cause blockage of the pores. It is a prerequisite to develop suitable microsphere beads with much greater size, i.e. >100 nm to allow the VLPs to diffuse in and out of the beads freely. We prepared gigaporous microsphere beads which are made of polystyrene, poly-glycidylmethacrylate and agarose respectively. The gigaporous media were found significantly superior to conventional agarose-based chromatographic media for macromolecules purification in many aspects. Besides high static and dynamic loading capacity, fast mass transport rate, the gigaporous structure also significantly improve the stability of the VLPs. For instance, the gigaporous media with pore size of 280 nm were successfully applied in chromatographic process for large-scale purification of rHBsAg, which loading capacity was increased 4-9 fold, bioactivity recovery was improved from 30% to 65%, and purification fold was raised from 1.3 to 2.7, compared with conventional media.
3. Multidimensional integrated protein chromatographic system and intelligent chromatography workstation

Long operating time is also one of the important causes for protein deactivation. Our group has constructed a new preparative workstation for simultaneous separation and purification of various proteins from a complex mixture. Various liquid chromatographic columns set up in the system can work sequentially, in parallel or in a mixed mode to enable simultaneous separation. This novel multidimensional integrated chromatography system has been successfully used in large scale production of homologous α-lactalbumin from 400 L transgenic bovine milk have completed 10 batches for Beijing Jipulin Biological Technology Co Ltd. Six kg qualified pure transgenic lactoferrin were obtained, which value for 4.5 million RMB per batch.

In the past 2 years, a new generation “intelligent protein chromatography system” (Fig. 4) with self-owned intellectual asset was developed and provided to more than ten companies in China.

4. Site-specific chemical modification and conjugation technology of proteins

Modification or conjugation of proteins with poly(ethylene glycol) (PEG), or other molecular like polysaccharide is one of the effective strategy to extending the circulation time of biopharmaceutical drugs in the body or increasing the immunogenicity of vaccines. The team has paved a way to prepare PEG modifiers with independent intellectual property rights, developed site-specific modification strategy, and efficient reaction and separation process for a variety of peptides, proteins and enzymes. The PEGylated rhG-CSF injection got clinical trial approval issued by State Food and Drug Administration (Approval No: 2011L01832). This PEGylated pharmaceutical possesses longer circulation time, better therapeutic effect and less amount of dose than other products.

Heterobifunctional PEG also was used as spacer arm to conjugate meningococcal capsular polysaccharide (PS) with tetanus toxoid to prepare a conjugate vaccine for prevention of life-threatening pathogen meningococcal diseases, 3.0-fold increase in the PS-specific IgG titers and a prolonged immune persistence was obtained.

5. Nanoscale biocatalyst systems for enzyme stability and multi-enzyme artificial cells

In vivo biocatalysis is one of the most important applications of bio-macromolecules. However, the bottleneck for their practical applications lies in the low activity and poor stability of enzymes in some of harsh environment like in organic
solvent system and the difficulties in coenzyme immobilization and its efficient regeneration. To tackle these challenges, nanoscale engineering have demonstrated to be an efficient strategy for smart biocatalysts with fine-tuned properties and functionalities. One successful example is the development of nanofibrous multi-enzyme artificial cells for complicated multi-step bio-transformation. Both enzymes and shared coenzyme are in-situ encapsulated inside the nano-chamber of the hollow-nanofiber via a facial co-electrospinning technique. The nano-confining effect provided the enzymes high activity and unique stabilizing mechanism, such that more than 170-fold increase in half-life at 25 °C was obtained for the encapsulated enzymes. The superiority of this hollow-nanofiber-based artificial cells to conventional discrete microsphere or microcapsules have been well demonstrated by several of complicated reaction systems, such as aqueous/organic bi-phasic interfacial reaction due to the unique interfacial-targeting properties, and aqueous/gaseous reaction due to high absorption capacity of the nanofiber membranes for gaseous reactants.

Fig. 7 Schematic illustrations of the setup for co-axial electrospinning (left), the nanofiber-supported multienzyme artificial cells involving 3a-HSD, DP and NAD(H) for bile acid assay (middle), and the stability of enzymes at 25 °C.

Selected Publications and Achievements

10. The First Prize of Association Award for Science and Technology from China Association for Instrumental Analysis (CAIA) : Chromatography Technology of Biotech Drugs purification Aiming at the Highest Bioactivity Protection
11. The First Class Award for Technological Invention from China Petroleum and Chemical Industry Federation. High performance purification of complex bio-macromolecules and its anti-denaturation technologies
Research Professors

**Prof. Zhiguo Su (Team Leader)** was born in 1954 and received his Ph.D. degree in biochemical engineering from University of Manchester, U K, in 1985. He was a postdoctoral fellow at the University of Delft, Netherlands (1986-1987). He has been a professor of IPE since 1997, where he had been the Director of National Key Laboratory of Biochemical Engineering (2001-2011) and Director of Legionary Key Lab. of Biopharmaceutical Process and Formulation Engineering since 2011. He is author of more than 260 scientific papers, co-author of 4 monographs, and held more than 140 patents including 4 international patents. He was awarded the Second Prize of National Technology Invention Prize (2009), the first prize of China Association for Instrument Analysis (CAIA) and China Petroleum and Chemical Industry Association (CPCIA). He won the National Science Fund for Distinguished Young Scholars in 1995.

**Affiliation**
Editorial Board, Artificial Cells, Blood Substitutes and Biotechnology
Editorial Board, Journal of Chemical Technology and Biotechnology
Editorial Board, Frontier of Chemical Science and Engineering
Editorial Board, Biotechnology and Applied Biochemistry

**Research Interests**
Purification and modification of peptide; Proteins; Vaccines and bioactive products; Refolding of protein; Preparation of blood/plasma substitutes.

**Prof. Tao Hu**, born in 1975, got his master degree in 1999 from Wuhan Institute of Virology, CAS, and then Ph.D. degree in Biochemical Engineering from Institute of Process Engineering, CAS in 2003. He was a postdoctoral fellow at Albert Einstein College of Medicine, New York, USA (2003-2008). He has been a professor of chemical engineering at IPE, CAS since 2008. He is mainly engaged in chemical modification of proteins. He is author of over 30 journal papers.

**Research Interests**
Polysaccharide conjugate vaccine; Tuberculosis subunit vaccine; Protein modification.

**Prof. Jingkai Yu** received his Ph.D. degree in Computer Science from Wayne State University (Detroit, MI) in 2006. He was a research associate at the Center for Molecular Medicine and Genetics, Wayne State University School of Medicine (2007-2009). He has been a professor at IPE since 2009, where he leads the Group of Bioinformatics and Molecular Simulation within the National Key Laboratory of Biochemical Engineering.

**Research Interests**
Building biological databases; Constructing & analyzing complex networks; Data analytics (data mining and machine learning); Molecular simulation for biological molecules; Network based drug discovery.
**Prof. Ruitian Liu** received his PhD in microbiology from Shandong University and completed postdoctoral work at Arizona State University. He is a professor of biopharmaceutics at Institute of Process Engineering, CAS. Research in the Liu’s lab focuses on engineering antibodies, vaccines and peptides for biomedical applications. By utilizing various biological engineering techniques, his work seeks to biopharmaceuticals to control important biological processes such as amyloid aggregation and cytotoxicity, hypertension, allergy, tumor formation, inflammation, and virus proliferation. Dr. Liu is developing antibodies, vaccines and peptides that can target amyloid oligomers or monomers of beta-amyloid, alpha-synuclein, Islet amyloid polypeptide (IAPP) and polyglutamine (polyQ) for the treatment of Alzheimer’s Disease (AD), Parkinson’s Disease (PD), Type 2 diabetes mellitus (T2DM) and Huntingdon’s Disease (HD). Current work is also focused on developing peptide vaccines and antibodies useful for treating hypertension, rheumatoid arthritis, cancer and viral infections. Some biopharmaceuticals are under investigation for their safety.

**Affiliations**
- Associate editor, Journal of Alzheimer’s Disease
- Associate editor, Advances in Bioscience and Biotechnology

**Research Interests**
- Biopharmaceutical investigation for the treatment of amyloidoses; Rheumatoid arthritis; cancer; Hypertension and viral infections.

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**Prof. Jianting Zheng** is a professor at the Institute of Process Engineering (IPE), CAS. He received his B.S. degree in biotechnology from Shandong University in 2001 and Ph.D. degree in biochemistry & molecular biology from Institute of Microbiology, CAS in 2007. He worked as a postdoctoral fellow at the University of Maryland, College Park (2008) and the University of Texas at Austin (2009-2013). He was recognized in 2014 as an awardee for the Recruitment Program of Global Youth Experts, China. He has published 16 papers in peer-reviewed journals in the related area.

**Research Interests**
- Structural and functional characterization of enzymes; Engineering of biosynthetic and regulatory pathway.

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**Prof. Yunshan Wang**, born in 1962, got his B. Sc in 1982 from Hebei University, and then Ph.D in 1998 from Graduate School of Chinese Academy of Agricultural Sciences. He worked as a post-doc and then research scientist at Institute of Process Engineering, CAS since 1998. He is mainly engaged in the scale-up and integration technology of microbial engineering and biotechnology products, including strains screening, fermentation engineering, separation and purification for protein, et al.
**Prof. Songping Zhang**, born in 1977, received her B. Sc in 1998 and then Ph.D in 2002, both from Tianjin University. Her doctoral dissertation received Nomination of National Outstanding Doctoral Dissertation Award, 2006 from the Ministry of Education of China. She worked as post-doc at Lund University, Sweden (2002-2004). After that, she worked at IPE, CAS, and became a professor in 2014. Her research encompasses the bio-catalysis engineering and bioseparation engineering. She has published about 50 peer-reviewed papers in highly cited journals, and held 6 patents.

**Research Interests**
Fabrication of novel nanofiber-based multienzyme system for biosynthesis and bioassay; Chemo-enzymatic synthesis of plant oil-based biodegradable polymer; Vaccine purification and preparation.