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Institut de Génie Chimique (IGC-IV): Laboratoire de Biotechnologie Cellulaire

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The Laboratory of Cellular Biotechnology is part of the Center of Biotechnology UNIL-EPFL. It addresses needs of academic and industrial research laboratories for more efficient technologies for the production and analysis of high-value recombinant proteins from animal cells cultivated *in vitro*. Animal cells and, in particular, immortalized mammalian cell lines have become the most frequently used production host systems for the synthesis of recombinant proteins which are applied in medicine for therapy or for vaccine purposes. The reason for this is, in spite of a somewhat elevated degree in complexity for processes based on mammalian cells, the ability of such cells to produce at high fidelity complex proteins which are large in size, have multiple glyco- and other modifications and require their protein processing machinery (golgi apparatus, secretory pathway, *etc.*).

Researchers in the laboratory are using equipment and technologies of molecular biology, cell biology, microbiology, mammalian cell culture technology, analytical and preparative chemistry, polymer chemistry, and chemical and process engineering. Modern techniques including capillary electrophoresis, HPLC, and biosensors are used at line for product characterization and bioprocess control. Also available is a top-of-the-art pilot scale facility for protein production and recovery from animal cells. Bioreactors range in scale from 2 to 100 l and appropriate downstream processing equipment for cell-product separation and protein purification allow the preparation of recombinant proteins in the milligram to gram range. This pilot scale laboratory is modeled after similar laboratories one finds in pharmaceutical companies which pursue the development and production of recombinant proteins for medical use.

A major effort in the laboratory is directed towards the establishment of novel principles and technologies for the **production of recombinant proteins in mammalian cells**. One of the research programs focuses on a new approach for protein expression in which the recombinant DNA molecules are transferred to cells which are already being cultivated at

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large scale. It is the goal of this program to synthesize, recover, and purify any desired protein in milligram to multigram quantities, starting from a DNA molecule, within 2–3 months. 'Standard' but not 'old' technologies as they have been developed within the last 10 years or so, would require 9 months or more to produce such quantities of protein. The alternative, faster technology, termed 'large scale transient expression' in mammalian cells is based on very recent developments (within the last 2 years) and requires, depending on the scale of operation for DNA transfer and subsequent protein production, significant quantities of plasmid DNA. Accordingly, a program is underway to not only optimize protein expression from mammalian cells, but also to improve plasmid production in *E.coli*. Linked to this is the need to recover and purify at high yield and with high-quality plasmid DNA from *E.coli* lysates derived from fermentations. Results from these efforts are useful and considered an important contribution to future DNA based therapeutic applications ('gene therapy').

Standard industrial technologies for the production of recombinant proteins in mammalian cells utilize Chinese Hamster Ovary cells, for various reasons. One of the reason for their popularity as industrial host systems is their ability to produce large quantities (up to 100 kg/year) of complex proteins when grown in suspension in large reactors (up to the 10000 l scale). Research efforts in the laboratory of *Cellular Biotechnology* are also directed towards the development of more efficient ways to establish highly productive stable cell lines and then to transfer such cell lines into an industrial modus. These efforts focus both on the molecular biology of vectors and their interaction with the chromatin of the mammalian host cell as well as the physiology, biochemistry, and physico-chemical interactions of cells cultivated under production conditions. The available bioreactor equipment in the laboratory allows both to mimic industrial scale operations as well as to develop new and improved processing principles for mammalian cell lines expressing a recombinant protein.

The development and implementation of novel strategies for the isolation and purification of the biotechnological high-value products produced in our facilities is another important research area of the laboratory. In this area, we concentrate on modern techniques such as affinity precipitation, selective membrane separators, displacement chromatography, *etc.* Whenever possible, the isolation methods are directly integrated into the overall production scheme, in order to improve the productivity and the safety of the bioprocess. Studies of the behavior of typical biotechnical products and key impurities (residual DNA/RNA, endotoxins, viruses, other proteins, *etc.*) are a major part of the development and optimization. For these studies, the most modern analytical and preparative equipment is available in the newly established laboratory. In addition a large effort is placed on the development of 'intelligent' synthetic polymers, which mimic certain aspects of biological molecules, *i.e.*, in their interaction with solids (protein displacers) or other biologicals (artificial antibodies, artificial affinity ligands, artificial enzymes), or aid their isolation (affinity macroligands, superadsorbers, thermoreactive membranes).

Product isolation increasingly becomes a bottle neck in modern biotechnology. This tends to be especially the case for the so-called high-value products, which are typically produced in rather low concentration in very complex fermentation environments. Concentration *cum* isolation is therefore the somewhat contradictory target. The use of affinity interactions early on in the downstream process becomes most attractive in this context. Certain biological molecules are able to identify and bind their respective counterparts even in the most complex biological environments. In **affinity precipitation** reversibly water-soluble polymers that contain such a product-binding domaine ('affinity macroligands', AML) are used to first capture and then co-precipitate the target molecule. Thus, affinity precipitation combines the high concentration factors of a precipitation technique with the high selectivity of an affinity technique, which makes this method extremely attractive for biotechnical downstream processing. The target-orientated design of specific AML, the engineering of affinity precipitation processes, *e.g.*, for the recovery of technical enzymes, as well as the basic study of the molecular and external factors contributing to the effect of the reversible precipitation of thermosensitive polymers are research topics in our group.

The later, high resolution stages of the downstream process of a recombinant bioproduct are usually dominated by 'chromatography', since the selectivity of this separation method is high enough to rid the product even of closely related impurities. The (nonlinear) elution chromatography on columns packed with porous particles, which currently predominates, however, may be less than optimal. **Displacement chromatography** is a noteworthy alternative. In displacement chromatography the separation is based on a displacer-enforced competition of the feed components for a limited amount of stationary phase binding sites. The feed mixture is resolved into consecutive, highly concentrated zones, the 'displacement train'. The capacity of the preparative column is used most efficiently. Several substances can be isolated at the same time, which makes this technique attractive for media recycling schemes in mammalian cell culture but also for the processing of sources containing more than one product such as (milk) whey, plasma, hemofiltrate, or plant extracts. Accessibility to the 'right' displacer is in displacement chromatography just as important as possession the 'right' stationary phase. The rational design of protein displacers has been addressed only recently, however, and displacer connected difficulties such as solubility, cost, toxicity, detectability, affinity, column regeneration, *etc.* tend to impede the application of the technique. Thus, the synthesis of protein displacers from a building block system containing several preactivated blocks, plays a major role in our efforts to use displacement chromatography in downstream processing. So far, several recombinant proteins have been isolated from cell culture supernatants and whey proteins from technical dairy whey.

Membrane adsorbers are thin-filter membranes that have been functionalized by the covalent linkage of interactive groups such as ion-exchange functions or affinity ligands. Given the all-or-nothing adsorption behavior which is typical for large biomolecules, such membranes are excellent stationary phases for biopolymer chromatography. There is, *e.g.*, hardly any internal mass-transfer limitation. The mobile phase passes convectively through the pores at high speed due to the low back pressure of the filters and the solutes have to cross only extremely short distances by molecular diffusion. Especially in the case of large biologicals, this becomes a huge advantage. Membrane-adsorber chromatography can be performed at throughputs which are several orders of magnitude higher than conventional columns chromatography. In the area of scale-up membrane adsorber chromatography profits from the large body of data accumulated in the area of filtration in general. Since several types of membranes can be assembled into a single module, mixed mode interaction chromatography is easily realized and controlled in membrane-adsorber chromatography.