# AC 2010-179: ILLUSTRATING BIOSEPARATIONS WITH THE PRODUCTION, PURIFICATION AND SEPARATION OF COLORFUL PROTEINS

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# Illustrating Bioseparations with the Production, Purification and Separation of Colorful Proteins


## Abstract

The field of chemical engineering is undergoing a rapid change. Advances in biology are prompting new discoveries in the biotechnology, pharmaceutical, medical technology, and chemical industries. Developing commercial-scale processes based on these advances requires that new chemical engineers clearly understand the biochemical principles behind the technology, in addition to developing a firm grasp of chemical engineering principles.[1] To ensure that chemical engineering students are prepared to contribute to these expanding industries, this project will incorporate hands-on and visually appealing experiments using colorful proteins to teach biochemical engineering and bioseparation principles.

The project consists of seven modules that introduce students to multidisciplinary engineering principles through the production and purification of colorful proteins. The project adapts experiments from the biochemistry and molecular biology education literature by expanding the scope from one colorful protein to many. Four colorful proteins with different physical properties will be mixed and separated using a variety of chromatography, ultrafiltration, and liquid-liquid (reverse micellar) separations, which will illustrate the basis of bioprocess design. To maximize student interest and learning, this material will be implemented in a hands-on and visually appealing format exemplifying the "hands-on / minds-on" approach to engineering education. The engineering goals of this project are:

- to explore bioseparation techniques
- to expose students to bioprocess design principles
- to study the performance of bioseparation processes using engineering principles
- to evaluate factors influencing the performance of bioseparation processes

To date, work has focused on chromatographic separation techniques. Learning modules on alternatives to chromatography will be presented.


## Introduction

Advances in biology are prompting new discoveries in the biotechnology, pharmaceutical, medical technology, and chemical industries. Developing commercial-scale processes based on these advances requires that new chemical engineers clearly understand the biochemical principles behind the technology, in addition to developing a firm grasp of chemical engineering principles.[1] To successfully deliver this knowledge to students, engineering educators require additional resources to illustrate relevant biological concepts throughout the curriculum.

This paper outlines the development of educational materials in protein production and several bioseparation techniques (chromatography, ultrafiltration, liquid-liquid extraction). In a typical bioprocess, the majority of the costs are associated with isolating and purifying the desired biological compound.[2] In many of the later stages of purification, over 50% use some type of

chromatography.[3] Exposing students to biochromatography provides an introduction to bioseparations and the underlying biochemistry concepts. Alternative bioseparation techniques can also be introduced, as a comparison to chromatography. As separation processes are based on the physical and chemical properties of the product and chief impurities, a wide range of concepts can be included, such as overall cell composition, protein biochemistry, recombinant protein production techniques, and bioprocess optimization.

These concepts can be introduced by improving undergraduate courses and laboratories through the development of exciting, visually-appealing experiments. The use of visually-appealing materials has been shown to motivate and captivate students in biology and chemical engineering settings.[4-9] Additionally, some elements of bioseparation (adsorption, ion-exchange, and chromatography) are difficult to teach in a lecture-based format, as these are rate-based, time-dependent processes.[10] These experiments will improve instruction in this difficult area by employing a range of colorful proteins with different biophysical properties.

To facilitate vertical integration in a variety of chemical engineering courses, two types of exercises have been developed. Demonstrations allow for these concepts to be introduced into lecture-based courses, and can be expanded to short hands-on exercises in small courses. Full-scale experiments allow for these concepts to be studied in more detail through unit operations and elective courses, where students can explore the effect of process parameters on separation efficiency. Both types of exercises are described in this paper.

**Results**

To date, three modules have been developed:

1. anion exchange chromatography with DsRed2 and EGFP[11]
2. anion exchange chromatography with EGFP and *C. anabaena* flavodoxin[12, 13]
3. protein production project with DsRed2, EGFP, and flavodoxin[14]

Separation of DsRed2 and EGFP by anion exchange is challenging, making this experiment sensitive to experimental conditions and suitable for extended experimentation. This anion exchange chromatography experiment is appropriate as a full-scale laboratory for unit operations laboratory courses and upper-level electives on biochemical engineering or bioseparations.

EGFP and flavodoxin can be separated easily with anion exchange chromatography, making this experiment fairly robust and suitable for demonstrations. This anion exchange chromatography experiment is appropriate for use as a demonstration in introductory engineering courses or courses focused on separation processes.

Full production and purification of the three colorful proteins makes for a thorough laboratory experience in upper-level electives on biochemical engineering.

Four more modules are currently in development:

1. cation exchange chromatography with horse heart cytochrome *c* and hen egg white lysozyme
2. batch ultrafiltration with cytochrome c and DsRed2

3. liquid-liquid extraction with cytochrome c in reverse micelles
4. liquid-liquid extraction to separate 2 or more proteins with reverse micelles

The cation exchange chromatography experiment was developed to ease adoption at other institutions. DsRed2 and EGFP (used in the anion exchange chromatography experiment) can be produced in the laboratory or purchased from commercial vendors. However, the production process requires some skill and time, and the proteins are expensive. To allow delivery of the same concepts, but ease adoption at other institutions, a cation exchange experiment using inexpensive proteins from commercial sources is being developed with cytochrome c and lysozyme. Similar to the first module, this cation exchange chromatography experiment is appropriate as a full-scale laboratory for unit operations laboratory courses and upper-level electives on biochemical engineering or bioseparations using proteins that are purchased, not produced.

Moving beyond ion exchange chromatography, an experiment focusing on hydrophobic interaction chromatography was not found to be successful. Other bioseparation techniques such as membranes and reverse micelles were then explored as alternatives to chromatography.

The batch ultrafiltration experiment is an excellent introduction to the separation of proteins by size using membranes. The proteins used, cytochrome c (12 kDa) and DsRed2 (103 kDa), have a large size difference and can be easily separated. Proteins of other sizes can be used depending on the availability of different disposable centrifugal ultrafiltration devices. For this module, a solution of cytochrome c and DsRed2 is placed in Amicon-Ultra membranes of different sizes, for example, 5 and 30kDa. The membranes are then centrifuged, allowing gravity to pull proteins smaller than the molecular weight cut off (MWCO) through the membrane pores. The retentate and filtrate are then analyzed by UV-visible spectroscopy and the concentration of both proteins in both phases can be determined from Beer's law. The results for both membranes can be compared to determine the best separation. This module is in early development but can be used as a laboratory assignment or hands-on exercise for a separations course or upper-level electives on biochemical engineering or bioseparations.

The extraction of proteins in reverse micelles is dependent of the conditions of the aqueous and organic phases, and the properties of the proteins. Changing these conditions can lead to a change in driving force (electrostatic or hydrophobic) and a change in extraction efficiency.[15-17] A simple extraction of cytochrome c into reverse micelles can be accomplished with an anionic surfactant, provided that appropriate conditions for the aqueous and organic phases have been choosen. This module is in early development also but can be used as a laboratory assignment or hands-on exercise for a separations course or upper-level electives on biochemical engineering or bioseparations.

A more complex extraction can be the separation of 2 proteins (any of cytochrome c, DsRed2, EGFP, or flavodoxin) by reverse micelles. This second reverse micelle module can be used as a demonstration, laboratory assignment, or hands-on exercise for separations course or upper-level electives on biochemical engineering or bioseparations.

The cation exchange chromatography experiment with cytochrome c and lysozyme and the reverse micelle experiment with cytochrome c can be easily adopted externally, as the proteins can be inexpensively purchased.

Results from the completed modules and modules in development will be presented on the poster.

## Summary

Three modules focused on the production, purification, and separation of colorful proteins have been developed. These modules have been successfully used in elective courses focused on biochemical engineering and core courses on separation processes. Four additional modules are currently in development, two of which should remove barriers to external adoption.

## Acknowledgements

The authors acknowledge funding from the National Science Foundation (NSF-EHR-DUE-CCLI 0633527).

### Bibliography

1. Lenhoff, A.M. (2003). A natural interaction: chemical engineering and molecular biophysics. AIChE J. 49, 806-812.
2. Lightfoot, E.N. and Moscariello, J.S. (2004). Bioseparations. Biotechnology and Bioengineering 87, 260.
3. Bonnerjea, J., Oh, S., Hoare, M., and Dunnill, P. (1986). Protein purification: the right step at the right time. Bio/technology 4, 954.
4. Ward, W.W., Swiatek, G.C., and Gonzalez, D.G. (2000). Green fluorescent protein in biotechnology education. Methods Enzymol. 305, 672-680.
5. Bes, M.T., Sancho, J., Peleaot, M.L., Medina, M., Gomez-Moreno, C., and Fillat, M.F. (2003). Purification of colored photosynthetic proteins for understanding protein isolation principles. Biochem. Mol. Biol. Educ. 31, 119-122.
6. Sommer, C.A., Silva, F.H., and Novo, M.R.M. (2004). Teaching molecular biology to undergraduate biology students. Biochem. Mol. Biol. Educ. 32, 7-10.
7. Hesketh, R.P., Slater, C.S., Farrell, S., and Carney, M. (2002). Fluidized bed polymer coating experiment. Chem. Eng. Ed. 36, 138-143.
8. Burrows, V.A. (2004). Experiments and other learning activities using natural dye materials. Chem. Eng. Ed. 38, 132-135, 141.
9. Komives, C., Rech, S., and McNeil, M. (2004). Laboratory experiment on gene subcloning for chemical engineering students. Chem. Eng. Ed. 38, 212-215, 221.
10. Wankat, P. (2001). Teaching separations: why, what, when, and how. Chem. Eng. Ed. 35, 168-171.
11. Lefebvre, B.G, Farrell, S., and Dominiak, R.D. (2007). Illustrating chromatography with colorful proteins. Chem. Eng. Ed. 41, 241-246.
12. Lefebvre, B.G. and Farrell, S. (2005). Illustrating bioseparations with colorful proteins. Proc of the 2005 ASEE An Conf and Expo, Portland, OR.
13. Cohen, T.M., Rohs, A.E., and Lefebvre, B.G. (2008). A laboratory demonstration illustrating bioseparations using colorful proteins. Biochem. Mol. Biol. Educ. 36, 287-291.
14. Lefebvre, B.G., Connell, L.E., and Dahm, K.D. (2008). Competition between student groups in the protein production challenge. Proc of the 2008 ASEE An Conf and Expo, Pittsburgh, PA.

15. Luisi, P.L., Giomini, M., Pileni, M.P., and Robinson, B.H. (1988). Reverse micelles as hosts for proteins and small molecules. Biochimica et Biophysica Acta. 947, 209-246.
16. Pires, M.J., Aires-Barros, M.R., and Cabral, J.M.S. Liquid-liquid extraction of proteins with reversed micelles. (1996). Biotechnol. Prog. 12, 290-301.
17. Melo, E.P., Aires-Barros, M.R., and Cabral, J.M.S. Reverse micelles and protein biotechnology. (2001). Biotech. Anu. Rev. 7, 87-129.