

# **AC 2008-2913: ILLUSTRATING BIOSEPARATIONS USING COLORFUL PROTEINS**

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# Illustrating bioseparations using 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.<sup>1</sup> 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 eight 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 and membrane 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 two chromatographic separation techniques. Learning modules on these two techniques 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.<sup>1</sup> 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 chromatographic separations. In a typical bioprocess, the majority of the costs are associated with isolating and purifying the desired biological compound.<sup>2</sup> In many of the later stages of purification, over 50% use some type of chromatography.<sup>3</sup> Exposing students to biochromatography provides an introduction to bioseparations and the underlying biochemistry

concepts. 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.<sup>4-9</sup> 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.<sup>10</sup> 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<sup>11</sup>
2. anion exchange chromatography with EGFP and *C. anabaena* flavodoxin<sup>12</sup>
3. protein production project with DsRed2, EGFP, and flavodoxin<sup>13</sup>

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.

Two more modules are currently in development:

1. cation exchange chromatography with horse heart cytochrome *c* and hen egg white lysozyme
2. hydrophobic interaction chromatography with DsRed2 and EGFP

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.

Moving beyond ion exchange chromatography, an experiment focusing on hydrophobic interaction chromatography is in development.

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. Two additional modules are currently in development, one of which should remove barriers to external adoption.

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