Module 1 overview

*lecture*
1. Introduction to the module
2. Rational protein design
3. Fluorescence and sensors

*lab*
1. Start-up protein eng.
2. Site-directed mutagenesis
3. DNA amplification

**PRESIDENT’S DAY**
4. Review & gene analysis
5. Protein expression
6. Purification and protein analysis
7. Binding & affinity measurements
8. High throughput engineering

4. Prepare expression system
5. Gene analysis & induction
6. Characterize expression
7. Assay protein behavior
8. Data analysis
Differences between prokaryotic and eukaryotic proteins sometimes require eukaryotic expression systems.

These two proteins exemplify characteristics that frequently call for eukaryotic expression:
## Prokaryotic vs. eukaryotic protein expression

<table>
<thead>
<tr>
<th>Property</th>
<th>Prokaryotic</th>
<th>Higher Eukaryotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>yield/(L culture)</td>
<td>1-100 mg</td>
<td>Widely variable</td>
</tr>
<tr>
<td>cost/(L medium)</td>
<td>~$5</td>
<td>~$50</td>
</tr>
<tr>
<td>Introduction of DNA</td>
<td>Transformation of competent cells</td>
<td>Viral or nonviral transfection</td>
</tr>
<tr>
<td>Handling</td>
<td>Sterile needles, etc.</td>
<td>Tissue culture hood</td>
</tr>
<tr>
<td>Cell incubation</td>
<td>Shaking incubator</td>
<td>Usu. w/CO₂-control</td>
</tr>
<tr>
<td>Induction</td>
<td>Usually IPTG</td>
<td>None, tetracycline</td>
</tr>
<tr>
<td>Glycosylation, etc.</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Notes</td>
<td>Best for small, globular proteins</td>
<td>Best for complex, eukaryotic proteins</td>
</tr>
</tbody>
</table>
Lecture 6: Protein purification

I. Standard purification methods
   A. Harvesting and lysis
   B. Protein separation techniques

II. Assessing purified proteins
   A. Electrophoresis
   B. Mass spectrometry
   C. Protein sequencing and AA analysis
Once we’ve collected the cells, how do we get the proteins out?
Separation techniques

most common, in addition to affinity

e.g. Ni-NTA

Nickel affinity purification with Ni-NTA agarose
Many other tags can be used for protein purification:

<table>
<thead>
<tr>
<th>tag</th>
<th>residues</th>
<th>matrix</th>
<th>elution condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly-His</td>
<td>~6Ni-NTA</td>
<td>imidazole</td>
<td>low pH</td>
</tr>
<tr>
<td>FLAG</td>
<td>8anti-FLAG antibody</td>
<td>low pH, 2-5 mM EDTA</td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>11anti-myc antibody</td>
<td>low pH</td>
<td></td>
</tr>
<tr>
<td>strep-tag8</td>
<td>modified streptavidin</td>
<td>2.5 mM desthiobiotin</td>
<td></td>
</tr>
<tr>
<td>CBP26</td>
<td>calmodulin</td>
<td>EGTA, EDTA</td>
<td></td>
</tr>
<tr>
<td>GST211</td>
<td>glutathione</td>
<td>reduced glutathione</td>
<td></td>
</tr>
<tr>
<td>MBP396</td>
<td>amylose</td>
<td>10 mM maltose</td>
<td></td>
</tr>
</tbody>
</table>

Tags may be chosen because they
- interfere minimally with protein structure/function
- improve recombinant protein expression or solubility
- offer most convenient purification methods

All tags may be cleaved from expressed proteins using specific proteases, if desired.
Gel filtration (size exclusion chromatography) principle

Quantification of purified proteins

use Beer-Lambert law:

\[ A_{280} = \varepsilon_{280} cl \]

\( \varepsilon_{280} \) is the extinction coefficient; it can be determined rigorously, or estimated:

\[ \varepsilon_{280} \sim n_W \times 5500 + n_Y \times 1490 + n_C \times 125 \]
Assessing proteins for identity and purity

Most standard technique is sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE):
- basis is the tendency of proteins to unfold in SDS and bind a fixed amount SDS per protein (1.4 g/g)
- negative charge of SDS overwhelms protein charges
- proteins have same charge to mass ratio, but are differentially retarded by the separation gel
- stacking layer “focuses” proteins before separation layer

http://nationaldiagnostics.com/article_info.php/articles_id/10
Coomassie brilliant blue staining

- binds proteins primarily via aromatic residues and arginine
- undergoes absorbance shift from 465 nm (brownish) to 595 nm (blue)
- basis for Bradford Assay; can be used to quantify proteins over ~3 kD

www.eiroforum.org/press/media_embl.html
SDS-PAGE gives an approximate MW and purity estimate, but how can we be sure the protein we’ve purified is the correct one?

- activity assay if one is available
- knowledge of exact mass (mass spectrometry)
- N-term. sequencing and AA analysis, if necessary

en.wikipedia.org/wiki/Mass_spectrometry
www.kcl.ac.uk/ms-facility/images/maldispec2.jpg
N-terminal sequencing (Edman degradation)

- products identified by chromatography or electrophoresis
- typically ~5 cycles practical for routine N-term. sequencing

en.wikipedia.org/wiki/Edman_degradation