Characterization of Recombinant Protein Reagents to Assure Quality

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ABRF Protein Expression Satellite Meeting 2007
What is the most important factor for success in recombinant protein projects?

Diplomacy

EVERYTHING should be discussed BEFORE the project is started

...and written down.
What Constitutes Quality?

Purity?
Quantity?
Solubility?
Activity?

What constitutes quality will vary for many projects!
<table>
<thead>
<tr>
<th>Different Labs</th>
<th>Different Views of Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Throughput QC Labs</td>
<td>Speed, Consistency, and Accuracy</td>
</tr>
<tr>
<td>Larger Academic Labs</td>
<td>Flexibility</td>
</tr>
<tr>
<td>Smaller Academic Labs</td>
<td>Fewer Personnel / Smaller Budgets</td>
</tr>
<tr>
<td>Structural Proteomics Labs</td>
<td>Purity, Solubility, High Concentrations</td>
</tr>
</tbody>
</table>

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Indicators of Quality:

Purity - Crystallography vs. Assay Reagent vs. Pharmaceutical

May need to Assess Protein Contaminants

May need to Assess Chemical Contaminants
Indicators of Quality:

Quantity and Solubility – These are often related

Can the protein be concentrated?

Will the protein remain soluble?

Can we add reagents to influence solubility?
  Sucrose
  Glycerol / Ethylene Glycol
  Detergents
Indicators of Quality:

Stability  -  How can we assess stability?

Does it remain soluble?

Does it maintain activity?  Who should assay?  How often?

Does it get oxidized?  Can we add antioxidants?

Can we store it?  Frozen?  Refrigerated?
Indicators of Quality:

Activity

Some assays are easy / Some are not!

Should the assay be done by the core lab, or by the customer?

Is activity reproducible? Try a few preps right from the beginning.
How Can We Assess Purity?

SDS-PAGE

“Home Made” Gels Are Cheaper.

Precast Gels Save Time and Can be Purchased in a Wide Range of Types.

- High and Low % Acrylamide
- Gradient Gels

Make Your Customer Pay for a Good Gel.

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How Can We Assess Purity?

MALDI-TOFF

Trypsin

GluC or CNBr

MALDI-TOF

MALDI-TOF
Develop a Good Report Form For MALDI-TOF Data

Guo-Hua Zhou et al.
World Journal of Gastroenterology
WJG, 1999 June; 5(3):235-240

Study on the quality of recombinant proteins using matrix-assisted laser desorption ionization time of flight mass spectrometry

### Table 3 MALDI-TOF-MS analysis of the tryptic digests of EPO

<table>
<thead>
<tr>
<th>rhEPO peptide</th>
<th>Position</th>
<th>Calculated mass value</th>
<th>Observed mass value</th>
<th>Relative mass value error (%)</th>
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<tbody>
<tr>
<td>T₀₁</td>
<td>1-4</td>
<td>439.5</td>
<td>440.5</td>
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<td>T₀₂</td>
<td>11-14</td>
<td>515.6</td>
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<tr>
<td>T₀₃</td>
<td>15-20</td>
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<td>T₀₄</td>
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<tr>
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<tr>
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<td>259.3</td>
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<td>*</td>
</tr>
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<td>-0.19</td>
</tr>
</tbody>
</table>

* A wide unresolved peak caused by T₀₁, T₀₃ and T₀₄.
What About MS Analysis of Whole Proteins?
What About MS Analysis of Whole Proteins?

ESI – Can be great if a protein is extremely pure and relatively small (<40Kd)

MALDI – Same as above

For MS analysis, salts, buffers, detergents, etc. can cause problems.

MS analysis of whole proteins is not practical for most labs as a routine analysis.
How Can We Assess Purity?
SDS-CE (Capillary Electrophoresis)
How Can We Assess Purity?

2D Gel Electrophoresis
How Can We Assess Purity?

HPLC

Ion Exchange can be very good, BUT, conditions must be optimized for each protein.

HPLC analysis fits best in labs that are high throughput and repetitive, where most of the proteins produced are similar in nature.
How Much Analysis is Too Much Analysis?
How Much Analysis is Too Much Analysis?
Thank you!

Questions?