The dynamic range of protein abundances

- Proteins exhibit ranges of concentration as much as ten orders of magnitude
- In plasma, albumin alone constitutes over 50% of the total protein (50 g/L; 0.8 mM)
- In cells, actin and tubulin, two structural proteins, predominate
- The challenge is study much lower abundance proteins such as DNA transcription factors (a few copies per cell) and cytokines (fM)
Remembering Avogadro

- 1 gram mole contains $6.02 \times 10^{23}$ molecules
- 100 fmol of a protein gives good coverage of its peptides - it contains $6.02 \times 10^{10}$ molecules
- How many cells do we need to have to analyze a given protein abundance?
  - 100 copies per cell $6 \times 10^8$ cells whole rat liver
  - 1000 copies per cell $6 \times 10^7$ cells 1 g rat liver
  - 10,000 copies per cell $6 \times 10^6$ cells 6 plates of cells
  - 100,000 copies per cell $6 \times 10^5$ cells 1 plate of cells
Simplifying the proteome

- Careful selection of a particular region of an organ - for example, the pituitary or hypothalamus in the brain
- Selection of a particular cell type using a cell sorting device, or laser capture methods (remember abundance)
- Subcellular fractionation for nuclei, lysosomes, mitochondria, peroxisomes, endoplasmic reticulum and cytosol
- Free flow electrophoresis to separate particulate organelles on the basis of their surface charge

PROTEIN PURIFICATION
How to study plasma

- Numerous companies have come out with products that systematically (but not completely) deplete plasma of albumin, $\gamma$-globulin, $\alpha_1$-antitrypsin, and transferrin using antibodies to these proteins coupled to beads.

- While this enriches the plasma for low abundance proteins, there is a risk that some of the latter are carried piggy-back on the proteins that were removed.
Properties of proteins that can be selected for chromatographically:

- Molecular weight - use of size exclusion columns
- Balance of positive and negative charges, i.e., exploiting the isoelectric point, pKa - ion exchange and chromatofocusing
- Hydrophobicity - salting out columns and reverse-phase LC
- Interactions with hydroxyapatite
- Specific sites recognized by affinity reagents
Chromatofocusing

Proteomics workshop
September 12, 2006
Purifying hBAT

- Separating a protein to ‘homogeneity’
  - By measuring the protein’s functionality (enzyme activity) or amount (by immunological methods - the latter do not necessarily test functionality)

DEAE anion exchange → chromatofocusing → Gel filtration → Pure protein
Purification of a protein with an affinity tag - in this example hBAT with a C-terminal Avi-tag

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>79.4</td>
</tr>
<tr>
<td>DEAE elution</td>
<td>74.7</td>
</tr>
<tr>
<td>Avidin flow through</td>
<td>12.7</td>
</tr>
<tr>
<td>Avidin elution</td>
<td>54.8</td>
</tr>
</tbody>
</table>

69% Percent Recovery

(1) Cytosol
(2) DEAE column elution
(3) avidin column flow through
(4) avidin column wash
(5) avidin column elution

Proteomics workshop
September 12, 2006
The future in proteomics

Chromatofocusing/reverse-phase LC

– Can work with larger amounts of protein (to 5 mg)
Capillary IEF/reverse-phase LC miniaturization keeps concentrations high
Is this the future?

Top-down analysis of proteins totally in the gas phase

– FT-ICR-MS with electron capture dissociation (ECD)
– Electron Transfer Dissociation (ETD)-MS in ion traps

But still requires protein purification up front

Thomas et al., J Prot. Res. 5:240, 2006

Proteomics workshop
September 12, 2006
Bibliography

• A talk on protein separation by Dr. Marilyn Niemann can be found at http://www.uab.edu/proteomics

• <click> on Class and look at the 2006 class schedule. There is a downloadable PDF file for the January 10, 2006 class.