Protein purification prior to proteomics analysis

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The dynamic range of protein abundances

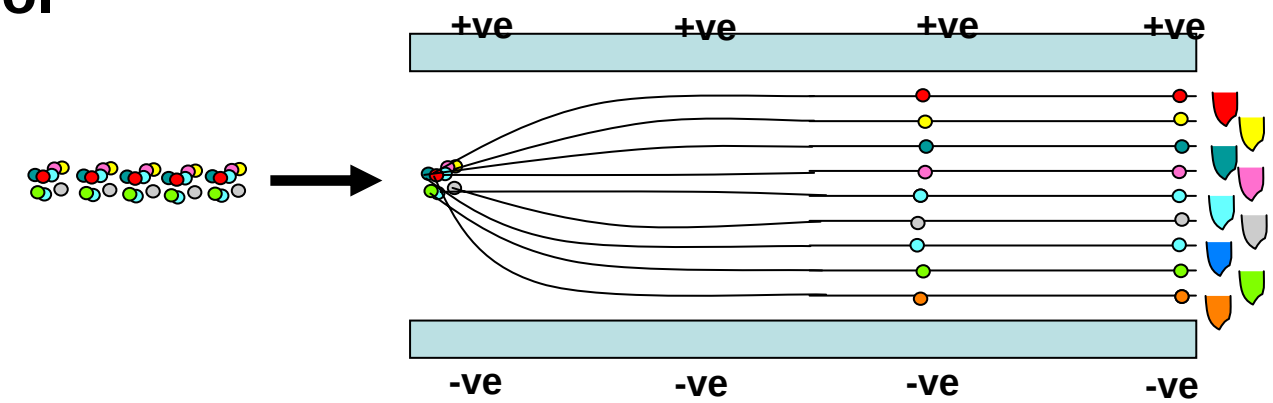
- **Proteins exhibit ranges of concentration as much as ten orders of magnitude**
- **In plasma, albumin alone constitutes over 50% of the total protein (50 g/L; 0.8 mM)**
- **In cells, actin and tubulin, two structural proteins, predominate**
- **The challenge is study much lower abundance proteins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

Remembering Avogradro

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

Simplifying the proteome

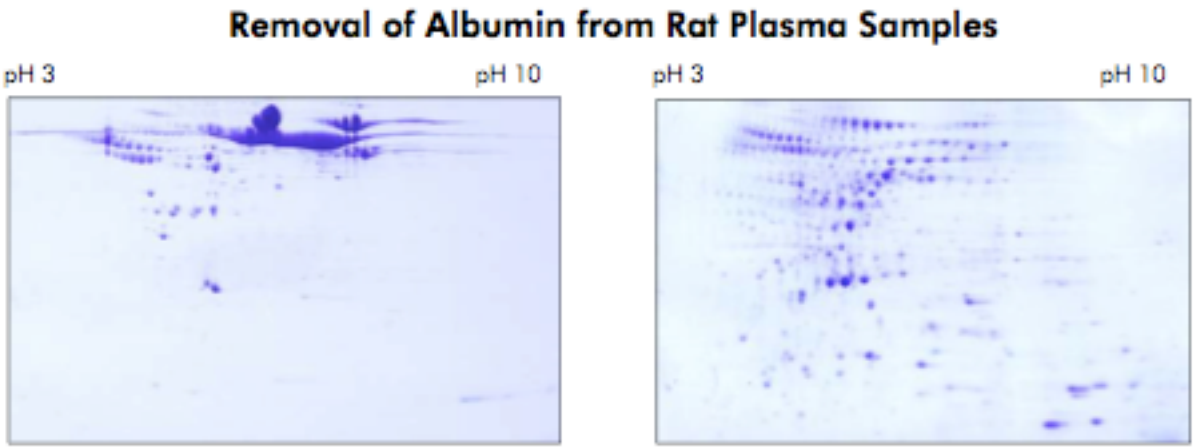
- Careful selection of a particular region of an organ - for example, the pituitary or hypothalamus in the brain
- Selection of a particular cell type using a cell sorting device, or laser capture methods (remember abundance)
- Subcellular fractionation for nuclei, lysosomes, mitochondria, peroxisomes, 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, γ-globulin, α_1-anti-trypsin, and transferrin using antibodies to these proteins coupled to beads**

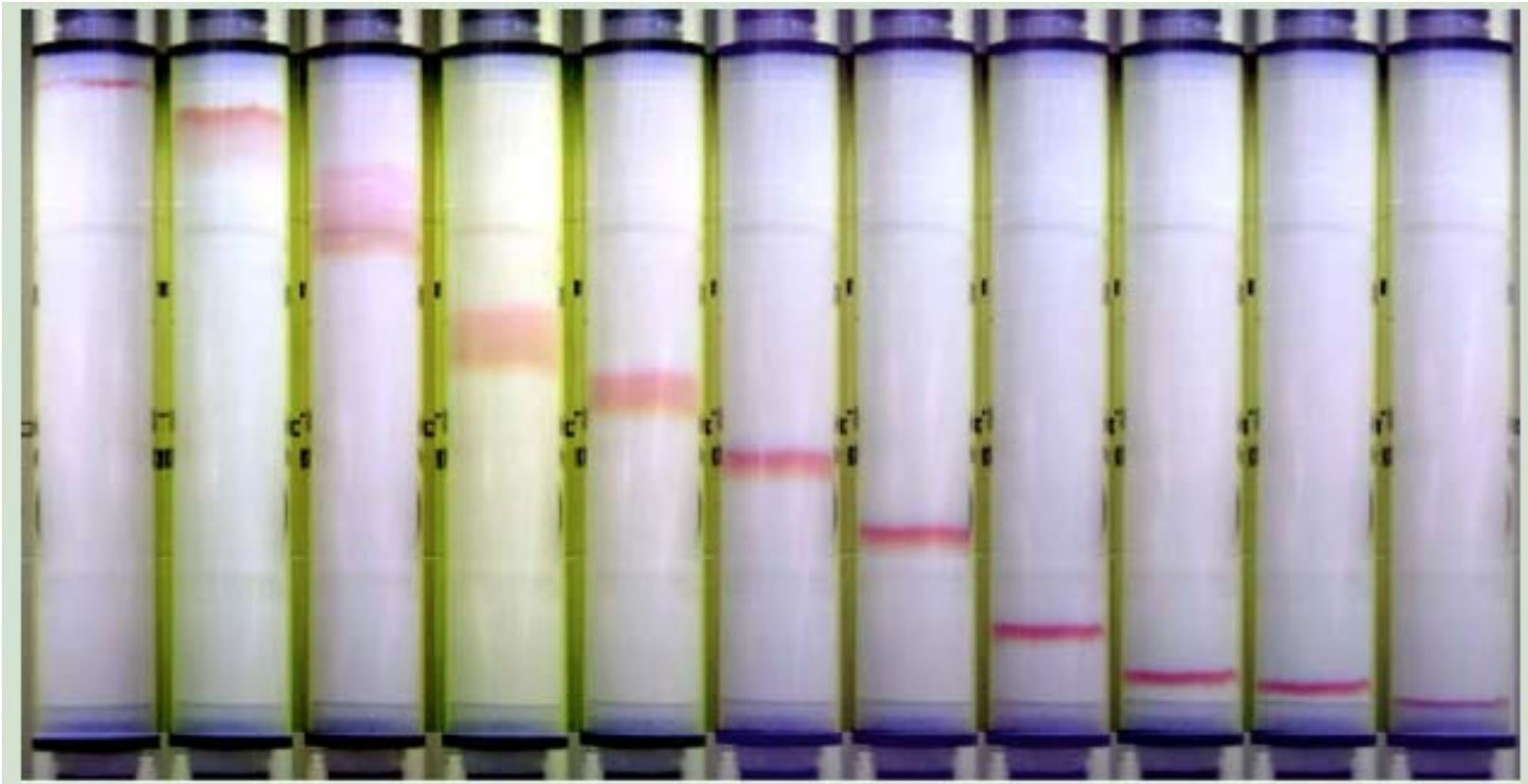


- **While this enriches the plasma for low abundance proteins, there is a risk that some of the latter are carried *piggy-back* on the proteins that were removed**

Properties of proteins that can be selected for chromatographically

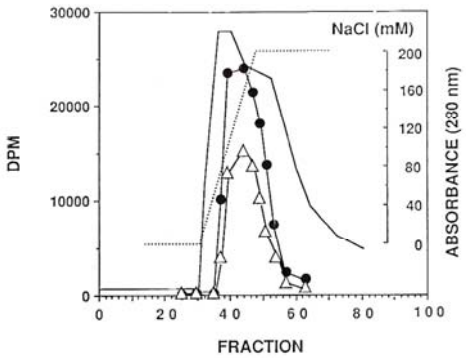
- **Molecular weight - use of size exclusion columns**
- **Balance of positive and negative charges, i.e., exploiting the isoelectric point, pKa - ion exchange and chromatofocusing**
- **Hydrophobicity - salting out columns and reverse-phase LC**
- **Interactions with hydroxyapatite**
- **Specific sites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

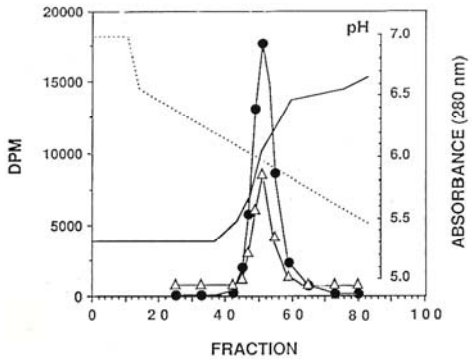
Chromatofocusing

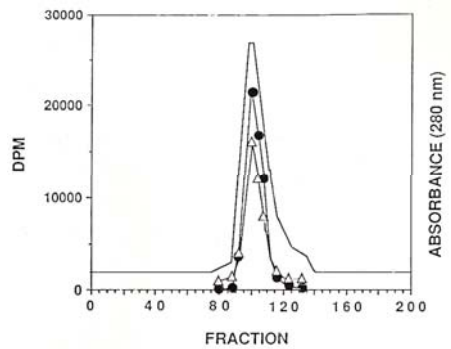


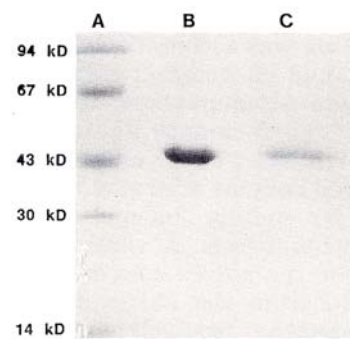
Purifying hBAT

- **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)**







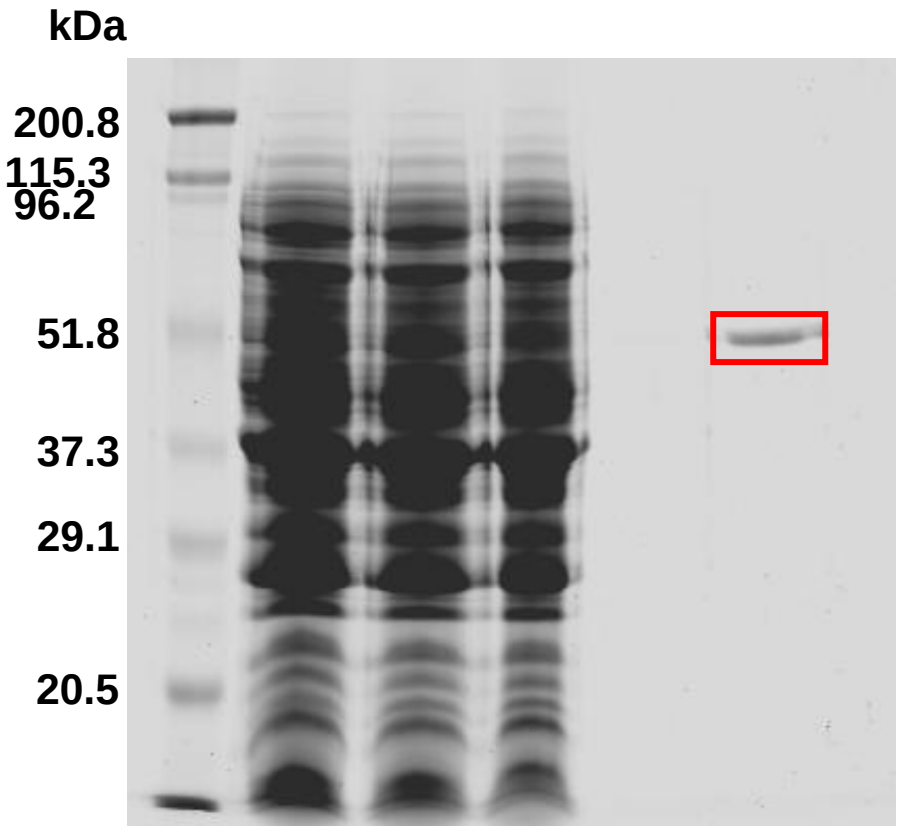


DEAE anion exchange → chromatofocusing → Gel filtration → Pure protein

Purification of a protein with an affinity tag - in this example hBAT with a C-terminal Avi-tag

Fraction	Total activity *(nmol/min)*
cytosol	79.4
DEAE elution	74.7
Avidin flow through	12.7
Avidin elution	54.8

69% Percent Recovery



(1) Cytosol

(2) DEAE column elution

(3) avidin column flow through

(4) avidin column wash

(5) avidin column elution

The future in proteomics

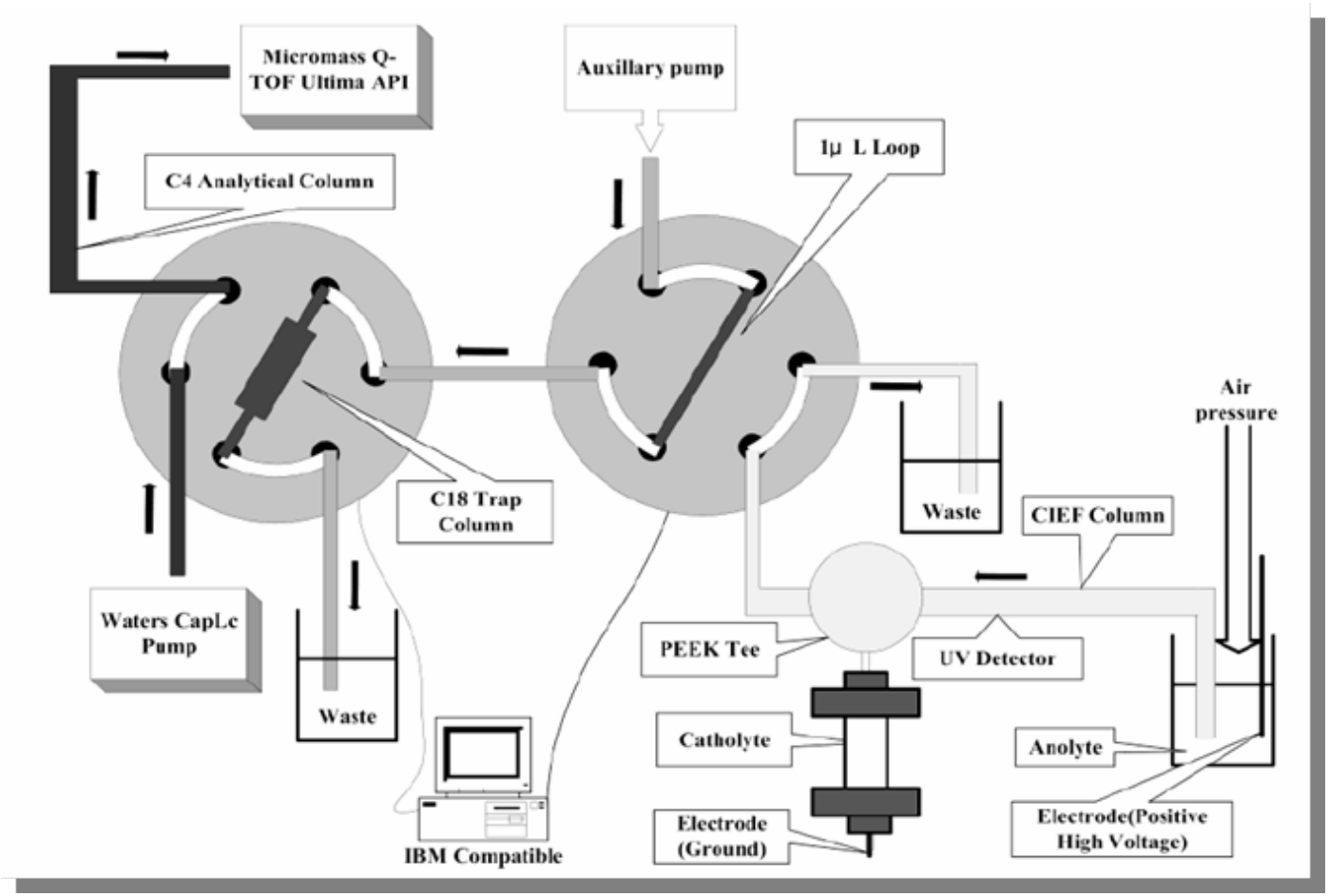
Chromatofocusing/reverse-phase LC

- **Can work with larger amounts of protein (to 5 mg)**

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Capillary IEF/reverse-phase LC

miniaturization keeps concentrations high



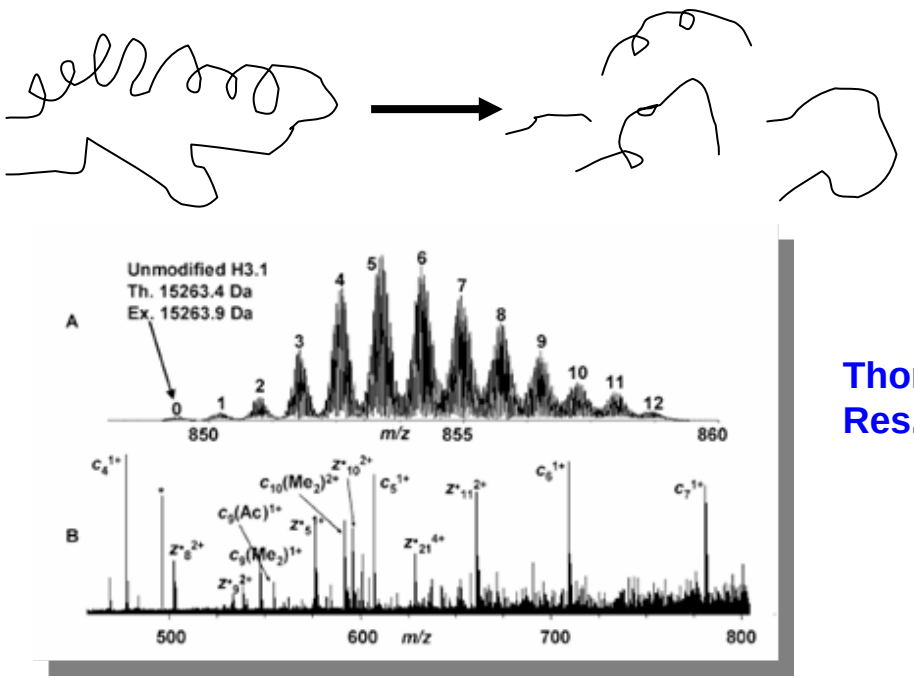
Zhou and Johnson, Anal Chem 75:2734, 2004

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

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.**