

## 2-D gels and protein mass spectrometry

1. Consider a 2-D gel separation of total cellular protein (isoelectric focusing versus SDS-PAGE, according to the Laemmli procedure, with SDS-PAGE separation running horizontally and isoelectric focusing vertically. When we examine such a gel, we often see vertical “streaks” consisting of several partly-separated protein spots spread out along the pI axis. We *don't* usually see horizontal streaks spread out along the MW axis. Why?

**Clusters of proteins with similar masses but distinct isoelectric points are common, due to post-translational modifications such as phosphorylation. Clusters of proteins with similar isoelectric points but distinct masses are less likely to be found.**

2. *Time-of-flight.* In a MALDI-TOF mass spectrometer, a protein molecule, mass 45 kDa, is desorbed by a very brief (3 ns duration) UV laser pulse and forms a +1 cation. The ion is accelerated across a voltage gradient of 1,000 V and then enters a drift tube of length 50 cm. How long will the ion take to reach the detector?

$$M = 4.5 \times 10^4 \times 1.66 \times 10^{-27} \text{ kg} = 7.5 \times 10^{-23} \text{ kg.}$$

$$q = e = 1.6 \times 10^{-19} \text{ Coul. } V = 10^3 \text{ Volts}$$

$$E = qV = 1.6 \times 10^{-16} \text{ J. } \therefore E = \frac{1}{2} mv^2 = 1.6 \times 10^{-16} \text{ J.}$$

$$\therefore v^2 = (2 \times 1.6 \times 10^{-16} \text{ J}) / (7.5 \times 10^{-23} \text{ kg}) = 4.3 \times 10^6 \text{ m}^2/\text{s}^2$$

$$\therefore v = 2 \times 10^3 \text{ m/s}$$

$$\therefore t = d/v = (0.5 \text{ m}) / 2 \times 10^3 \text{ m/s} = 2.4 \times 10^{-4} \text{ s} = 0.24 \text{ ms}$$

3. *Exact masses.* By definition, the mass of the  $^{12}\text{C}$  atom = 12.0000 amu (atomic mass units). However, the atomic weight of carbon, listed on the periodic table, is 12.011.  
a) Account for this difference.

**The atomic weight (mass) is the weight (mass) of Avogadro's number of atoms. Natural carbon is comprised mainly of the isotope  $^{12}\text{C}$ , with an admixture of 1.107%  $^{13}\text{C}$ , mass = 13.00335. The atomic weight is therefore a weighted average of the weight of the two isotopes.**

b) The mass spectrum of methane ( $\text{CH}_4$ ) shows a molecular ion peak ( $\text{M}^+$ ) at  $1 \times 12 + 4 \times 1 = 16$  (the “base peak”); another peak, with about 1% of the intensity of the base peak, occurs at mass 17. Explain the presence of the latter peak.

**The mass-17 peak is due to  $^{13}\text{CH}_4$  and  $^{12}\text{C } ^1\text{H}_3 \text{}^2\text{H}$ .**

c) In protein mass spectrometry, however, the mass spectrum usually consists of one broad peak, centred at a mass corresponding to the *atomic weight* of the protein, as calculated from the periodic table's atomic masses. For example, consider a hypothetical protein with composition:  $\text{C}_{601}\text{H}_{723}\text{N}_{126}\text{O}_{230}$ . The observed peak mass is  $601 \times 12.011 + 723 \times 1.0079 + 126 \times 14.0067 + 230 \times 15.9994 = 13,392$ . (By contrast,  $601 \times 12.000 + 723 \times 1.007825 + 126 \times 14.00305 + 230 \times 15.9949 = 13,384$ .) Explain why the calculation for proteins must be done differently from the calculation for small molecules.

**The natural abundance of  $^{13}\text{C}$  is only about 1%, so, for compounds with a small number of carbon atoms, few molecules will contain any heavier isotopes of C (or most other elements). The difference in mass between M and M+1 is a significant fraction of M, so the peaks are easily resolved by the spectrometer. But proteins contain hundreds or even thousands of carbon atoms, so almost all molecules will contain many heavier isotopes, as dictated by binomial statistics. The experimentally-observed peak is, therefore, the sum of dozens of component peaks, each corresponding to a particular isotopic composition (exact mass). The difference in mass between M and M+1 is, in this case, a very small fraction of M (e.g., 1 part in 50,000 for a 50 kDa protein), so the peaks are probably not resolved. A bell-shaped distribution of masses will be observed, centred at the most likely mass, which is the statistical average represented by the (macroscopic) atomic weight value.**

4. Most of the amino acids found in proteins have diagnostic masses. However, one pair has identical masses. Which pair is that? **Leucine and isoleucine.**

Another pair has masses which differ by only 0.03%. Which pair is that?

**Lysine:  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}$ : monoisotopic mass = 128.09496**

**Glutamine:  $\text{C}_5\text{H}_8\text{N}_2\text{O}_2$ : monoisotopic mass = 128.05858**

How do these facts affect protein sequencing by mass spectrometry?

**These pairs cause ambiguities (I/L and K/Q) in interpretation of peptide composition based on mass alone.**

5. CcrA3 is a  $\beta$ -lactamase (an enzyme that degrades  $\beta$ -lactam antibiotics, such as penicillin) from the bacterium *Bacteroides fragilis*. The crystal structure of CcrA3 shows that the active site of this enzyme contains a binuclear Zn center. Researchers at Wyeth-Ayerst Research (Yang Y., *et al.*, *J. Biol. Chem.* 274: 15706-15711, 1999) studied recombinant CcrA3 expressed in *E. coli*. Electrospray mass spectrometry was used to study metal binding to the enzyme.

Explain the chemical difference which accounts for the observed mass difference between the native and denatured enzymes.

**Native: 25372.0      Denatured: 25247.4      difference = 124.6**

**Zn isotopes**

<b>nom.</b>	<b>exact</b>	<b>abund.</b>
<b>64</b>	<b>63.929</b>	<b>48.63%</b>
<b>66</b>	<b>65.926</b>	<b>27.90%</b>
<b>67</b>	<b>66.927</b>	<b>4.10%</b>
<b>68</b>	<b>67.924</b>	<b>18.75%</b>
<b>70</b>	<b>69.925</b>	<b>0.62%</b>

**average mass = 65.4      2 × Zn = 131**

**Two zinc ions are bound in the native state and lost upon denaturation. The difference in mass between the native and denatured proteins is about 125, which is equal to gain of two zinc atoms, in the form of Zn<sup>2+</sup>, accompanied by loss of 6 mass units -- presumably about 6 H<sup>+</sup> ions which dissociate from the protein, concomitant with zinc binding.**