

## Exercise Sheet 7 – Computational mass spectrometry

### Introduction to mass spectrometry

#### 1. Mass spectrometry (5.0 points)

a) Name the core components of a mass spectrometer and describe their function briefly (max 4 sentences). (3 points)

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**Ion source:** ionizes and vapourizes the analytes into the vacuum of the mass spectrometer (e.g. electrospray or MALD)

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**Mass analyzer:** induces differences in flight path of the ion dependent on their  $m/z$  ratio by e.g. deflecting them in a magnetic field or trapping them (orbitrap).

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**Detector:** detects the current induced by the deflected ions or counts them.

Can be e.g. a Faraday cup, a microchannel plate or (for orbitraps) metal strips for ion current detection.

a) Explain concisely (in 2 sentences) what a mass spectrum is and what information it depicts. Use the following terms:  $m/z$ , intensity, peak(s). (2 point)

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A mass spectrum is a plot of  $m/z$  vs intensity that plots the intensity of the signal detected by the mass spectrometer for each  $m/z$  value (often as a count of detected particles).

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Peaks in the spectrum correspond to fragments of a certain mass at a given ionization level; depending on the resolution, peaks may be resolved into multiple subpeaks for each isotopic composition of the fragment.

## 2. Peptides, fragmentation, peaks and masses (10.0 points)

a) Write a small program/script/function (language of your choice) which calculates and returns all possible tryptic peptides for any protein sequence. The function may look similar to "String[] getTrypticPeptides(String proteinSequence)". Provide your source code and its output (as PDF at the end) and the number of peptides generated (fill in here) for the protein P005331. Download the protein sequence from UniProt.org. (4.0 points) You will reuse the source code in the next exercise.

Number of tryptic peptides: 828 (peptides above 500 Da, 12722 without average filter)

b) Write a small program/script/function (language of your choice) which calculates and returns the monoisotopic masses of all possible singly charged y and b fragment peaks with at least 7 significant figures for any given input peptide. The function may look similar to "double[] getFragmentMasses(String sequence)". Provide your source code and its output (as PDF at the end) and the output for the peptide "MAINHTGEK" (fill in table). (4.0 points) You will reuse the source code in the next exercise.

b+	m/z	AAS	m/z	y+
1	150.05879	M	1000.4885	9
2	221.0959	A	869.4480	8
3	334.1800	I	798.4108	7
4	448.2229	N	685.3268	6
5	585.2818	H	571.2839	5
6	686.3295	T	434.2250	4
7	743.3509	G	333.1773	3
8	872.3935	E	276.1558	2
9	1000.4885	K	147.1132	1

b) The first isotope (peak) of ethanol ( $C_2H_5O_1H_1$ ) contains 1 additional neutron. Let's assume that the neutron is either incorporated in form of a heavy H ( $^2H$ ) or heavy C ( $^{13}C$ ). Assume you pick two molecules from the first isotope, one containing a single heavy H ( $C_2H_5O_1^2H_1$ ) and the other containing

a single heavy C ( $^{13}\text{C}_1\text{C}_1\text{H}_5\text{O}_1\text{H}_1$ ). The difference between the two molecules is which atom contains the additional neutron. With the information at hand, what do you expect the mass of  $^{13}\text{C}_1\text{C}_1\text{H}_5\text{O}_1\text{H}_1$  to be in relation of  $\text{C}_2\text{H}_5\text{O}_1^2\text{H}_1$ ? Smaller, equal or bigger? Explain your reasoning (1 sentence). (2.0 points)

X  $^{13}\text{C}_1\text{C}_1\text{H}_5\text{O}_1\text{H}_1$  is lighter (smaller m/z) than  $\text{C}_2\text{H}_5\text{O}_1^2\text{H}_1$

$^{13}\text{C}_1\text{C}_1\text{H}_5\text{O}_1\text{H}_1$  is equal (same m/z) than  $\text{C}_2\text{H}_5\text{O}_1^2\text{H}_1$

$^{13}\text{C}_1\text{C}_1\text{H}_5\text{O}_1\text{H}_1$  is heavier (bigger m/z) than  $\text{C}_2\text{H}_5\text{O}_1^2\text{H}_1$

Explanation:

The mass difference between  $^{13}\text{C}$  and  $^{12}\text{C}$  is  $13.0033 - 12.0000 = 1.0033$ ; the mass difference between deuterium and protium is  $2.0141 - 1.0078 = 1.0063$ .

~~We would expect the mass difference to be equal in ethanol, as the molecular environment~~  
does not significantly affect the nuclear binding energy.

Therefore, deuterated ethanol is heavier than ethanol substituted with  $^{13}\text{C}$ .