Task 1

ICE

9th of May 2017

COUNTRY:

Team:
General Instructions

You have 4 hours to complete the following tasks.

Wear laboratory coat and protective goggles at all times in the laboratory.
   If you wear glasses already this is sufficient.
Eating and drinking is prohibited in the laboratory.

If you have skin contact with chemicals rinse immediately with tap water.

All paper used, including notes, must be handed in at the end of the experiment.

All results must be entered into your answer sheet.

Your graphs must be handed in along with the answer sheet.

Only the yellow answer sheet, and the attached graphs, will be marked.

The Task consists of 4 experiments.

   Experiment 1: 32 marks
   Experiment 2: 32 marks
   Experiment 3: 20 marks
   Experiment 4: 26 marks
Introduction

Welcome to the Ice Lab. This is going to be your place of work during the next few hours. You are going to investigate the climate and conditions of life in the past.

The increasing amount of greenhouse gases in the atmosphere is changing the climate of the Earth in a decisive way. Experts are engaged in lively discussions about the expected increase in the global mean temperature and whether it will give rise to a violent melting of the polar ice sheets. Among these is the inland ice of Greenland. Such a melting could give rise to increases in the level of the oceans of many meters.

If we are to be able to predict the future climate, there is a pressing need to improve the knowledge and understanding of the climate of the past. One of the tools for this is the investigation of ice cores, samples of the ice cap, drilled from the surface down to the solid rock beneath. In Greenland, the thickness of the ice cap is in some places more than 3000 m. Ice cores represent a source of comprehensive knowledge of the climate in the past. Measurements of the properties of the ice and its content of impurities and air bubbles allow one to investigate the atmosphere, seas and ice caps of the past with a high amount of details.

You are going to investigate ice core samples from the test station NEEM in Greenland, and you will be analysing DNA-fragments in samples from the Dye-3 position. Also, you will determine recent living organisms from so-called cryoconite holes in the arctic ice. You will characterize the climate in earlier times from physical, chemical and biological examinations.

In this way, you can contribute decisively to the understanding of the climate in the present and in past interglacial periods, and thus improve on the understanding of the dynamics of the climate system and on the possibility to predict the reaction of the ice caps to future climate changes.

Figure 1.0. Greenland, located close the north pole. The localizations of the drilling spot NEEM and the Dye-3 position are shown.
Experiment 1 32 marks

Introduction

Polar ice cores can be used to provide valuable information on past climate. Willi Dansgaard, a pioneer in polar ice core research, proposed in 1952 that the ratio of the heavy to the light isotopes of oxygen in the water molecules of polar precipitation shows a strong correlation with the temperature of the atmosphere at the time of the snowing event. As a result, study of ice from the deeper layers of the Greenland and the Antarctic ice caps can yield information on past climate spanning tens to hundreds of thousands of years back in time.

Here you will have a unique opportunity to perform measurements and calculations using real ice core samples from the Greenland ice cap. First, you will perform measurements of ice density and later you will reconstruct past Greenland temperatures, using state of the art laser spectroscopy measurements.

Materials

- 1 cylindrical ice core sample, 2 cm thick
- 1 beaker, 1000 mL
- 1 bottle of cold, deionized water
- 1 precision laboratory scale (+/- 0.1 g)
- 1 potato fork
- 1 thermometer
- 1 plastic calliper
- Millimetre paper
- 2 bottles with samples of water from ice core – will be handed out
- 2 plastic pipettes
- 2 vials

1.1 The density of ice

The top layer of Greenland's ice consists of firn. With the term firn (in German it literally means snow of the last year) we refer to the material that is going through a densification process from snow to solid ice. The first part of this assignment is the experimental determination of the density of a real ice core sample from the Greenland ice cap.
Please pay attention to the following:

The following experiment requires that you work with a piece of ice in an environment with temperature above zero °C. As a result, you are advised to prepare your equipment, read carefully the assignment steps in advance and make a plan as a team before you fetch the ice core sample from the freezer. Extensive delays during this experiment may result in unnecessary melting of the ice core sample and thus poor measurement results.

**Question 1.1**

Work through the following steps.

**Question 1.1.1**

a. Fetch the beaker and cold deionized water from the fridge and fill the beaker with 0.5 L water. Measure the temperature of the water $T_w$.

➢ Write down the result in the answer sheet, box 1.1.1.

b. Using the plot provided in Figure 1.1, find the density of the water $\rho_w$ for the temperature you measured.

➢ Write down the result in the answer sheet, box 1.1.1.

![Figure 1.1. Density of water as a function of temperature.](image)

**Question 1.1.2**

c. Place the beaker on the scale and measure the mass of the beaker and the water $m_{w+b}$.

➢ Write down the result in the answer sheet, box 1.1.2.
d. Fetch the ice core sample from the freezer.
e. Using the plastic calliper measure the diameter $D_{\text{ice}}$ and the thickness $H_{\text{ice}}$ of your sample.
   - Write down the result in the answer sheet, box 1.1.2.
f. Place the ice sample in the water. Measure the new mass $m_{g+w+\text{ice}}$.
   - Write down the result in the answer sheet, box 1.1.2.
g. Apply force with the fork to submerge the ice. Record the new reading $m_{g+w+\text{ice}+\text{force}}$.
   - Write down the result in the answer sheet, box 1.1.2.

Question 1.1.3
h. Calculate the volume of your sample $V_{\text{ice}}$.
   - Write down the result in the answer sheet, box 1.1.3.
i. Calculate the mass of the ice core sample $m_{\text{ice}}$.
   - Write down the result in the answer sheet, box 1.1.3.
j. Use the results of steps h and i to calculate the density of the ice $\rho_{\text{ice}}$.
   - Write down the result in the answer sheet, box 1.1.3.
k. Use the results from steps g and i to give a second estimate of the ice density $\rho'_{\text{ice}}$. Show the intermediate steps in your calculation.
   - Write down the result in the answer sheet, box 1.1.3.

Question 1.1.4
In this question, uncertainties of some of the measurements will be estimated using min-max method.
l. Calculate the difference $\rho'_{\text{ice}} - \rho_{\text{ice}}$.
   - Write down the result in the answer sheet, box 1.1.4.
m. You have probably observed that the reading you get for $m_{g+w+\text{ice}+\text{force}}$ when the ice is submerged is somewhat unstable. Give an estimate of the uncertainty $\Delta m_{g+w+\text{ice}+\text{force}}$.
   - Write down the result in the answer sheet, box 1.1.4.
n. Based on your answer in step m, give an estimate of the uncertainty $\Delta \rho'_{\text{ice}}$.
   - Write down the estimate in the answer sheet, box 1.1.4.
Assuming the quality of the cutting of the sample and the calliper measurement result in an uncertainty of 0.2 mm for both $D_{\text{ice}}$ and $H_{\text{ice}}$, calculate the uncertainty of the estimate of $V_{\text{ice}}$ and $\rho_{\text{ice}}$ ($\Delta V_{\text{ice}}, \Delta \rho_{\text{ice}}$).

Write down the result in the answer sheet, box 1.1.4.

1.2 Densification of snow to ice and pressure in the ice

The pressure needed to force the firn densification process comes from the weight of the material itself. As a result, the density increases with increasing depth as shown in Figure 1.2. The pressure at a depth $z$ is given by the equation:

$$p(z) = \frac{g \cdot m_z}{A}$$

where $g$ is the gravitational acceleration (9.81 ms$^{-2}$) and $m_z$ is the mass of a column of overburden ice at depth $z$ and with cross sectional area $A$.

Question 1.2.1

Based on the pressure equation and Figure 1.2, answer if the following sentences are true or false.

<table>
<thead>
<tr>
<th>Statement</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p(z)$ depends on only the depth $z$.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At sufficiently large depths, the pressure $p(z)$ is roughly a linear function of depth.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p(z)$ depends on the depth $z$ and the density $\rho(z)$.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p(z)$ is independent of depth $z$.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p(z)$ is independent of the cross-sectional area of the column $A$.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tick your answers in the answer sheet, box 1.2.1.

Question 1.2.2

Estimate the pressure and density of the ice at the following depths using Figure 1.2.

<table>
<thead>
<tr>
<th>Depth $z$ [m]</th>
<th>Density $\rho$ [kgm$^{-3}$]</th>
<th>Pressure $p$ [kPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Give your answers in the answer sheet, box 1.2.2.

Our small drilling machine can retrieve ice cores with a diameter of 74 mm and a length of 1 m from depths up to 350 m.

Question 1.2.3

Calculate the mass of a drilled ice core from these depths.
The big drilling machine can retrieve ice cores with a diameter of 98 mm and a length of 4 m at depths reaching the bottom of the ice cap.

**Question 1.2.4**

Calculate the mass of an ice core drilled with the big drilling machine at these depths.

- **Give your answers in the answer sheet, box 1.2.4.**

<table>
<thead>
<tr>
<th>Depth $z$ [m]</th>
<th>Mass $m$ [kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
</tbody>
</table>
1.3 Dating of ice and isotopes

For the next part of this experiment you will be working with ice core samples from the deeper part of the NEEM ice core. The NEEM ice core was drilled in North-East Greenland between 2007 and 2012 and contained ice from the previous warm interglacial time.
You will be given two bottles with real ice core samples from two different depths of the ice sheet. You will be asked to estimate the age of both samples, calculate the temperature over the ice sheet and in the end, you will be asked to prepare sample vials in order to perform isotopic analysis on a laser spectrometer.

Ice flow

We normally think of ice as a solid material. In reality, ice is a material that under stress can behave like a fluid and no other place in the world presents a better example of this behaviour than the ice caps of Greenland and Antarctica. Each year a new layer of snow falls on the ice cap. After an annual
layer of snow has undergone a process of densification, the load of the overburden ice results in a continuous stretching and thinning of the ice layers. As shown in Figure 1.3, an ice layer with an initial thickness of 20 cm (typical for the case of NEEM for present conditions) undergoes a thinning process that has the effect that at a depth of 1000 m the same layer has a thickness of about 10 cm (50% of the initial value).

By using very precise and high-resolution measurements of water isotopes and chemical impurities, we are able to measure the thickness of the annual layers in the ice core. In the following table, you can see what a year’s layer thickness (let us call it $\lambda$ from now on and express it in meters per year) looks like, based on real measurements of ice from the NEEM ice core.

**Question 1.3.1**

Based on the data present in Table 1, make a graph of $\lambda$ as a function of depth $z$ on the millimetre paper you are given. Label the graph “Graph 1.3.1” and attach it to the answer sheet.

> Attach “Graph 1.3.1” to the answer sheet.

<table>
<thead>
<tr>
<th>Depth $z$ [m]</th>
<th>Annual Layer Thickness $\lambda$ [m/yr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>500</td>
<td>0.20</td>
</tr>
<tr>
<td>1200</td>
<td>0.10</td>
</tr>
<tr>
<td>1400</td>
<td>0.04</td>
</tr>
<tr>
<td>1500</td>
<td>0.0125</td>
</tr>
</tbody>
</table>

**Age of the ice**

The quantity $\lambda$ is extremely useful as it can be used in order to calculate the age of a layer of ice. If $\lambda$ is known as a function of depth and we assume a thin layer with thickness $\Delta z$, then the number of years included in this layer $\Delta t$ will be given by the equation:
\[ \Delta t = \frac{1}{\lambda} \cdot \Delta z \]

For a thicker layer between for example the depths \( z_1 \) and \( z_2 \) the number of years \( t_2 - t_1 \) will be given by the area under the \( 1/\lambda \) curve as shown in Figure 1.4.

![Figure 1.4](image)

**Question 1.3.2**

Use Table 1 to calculate the quantity \( 1/\lambda \) at the depths of 0, 500, 1200, 1400 and 1500 m. Plot the quantity \( 1/\lambda \) as a function of \( z \). Connect the points with straight lines in order to ease later calculations. Label the graph “Graph 1.3.2” and attach it to the answer sheet.

➢ Attach “Graph 1.3.2” to the answer sheet.

**Question 1.3.3**

The graph you created in 1.3.2 can help you calculate the age of the ice at the given depths. Calculate the age of the ice for \( z = 0, 500, 1200, 1400 \) and 1500 m (by the term age we mean the total number of years from the surface and until the depth of interest). Create a new graph (“Graph 1.3.3”), where you present the age \( t \) as a function of depth \( z \).

➢ Attach “Graph 1.3.3” to the answer sheet.

**Water isotopic ratios in polar ice**

Chemical elements with the same number of protons but a different number of neutrons are called isotopes. The water molecule \( \text{H}_2\text{O} \) can be found in nature in different variants containing different isotopes. The most common water molecule contains \(^1\text{H}\) and \(^{16}\text{O}\) (where superscripts 1 and 16 denote the number of nucleons), while the two second most common variants are \(^1\text{H}\)^{18}\text{O}\(^1\text{H}\) and \(^1\text{H}\)^{16}\text{O}.
These molecular variants are called isotopologues. Isotopologues behave identically with respect to their chemical properties, but due to their mass difference, they differ with respect to physical properties, like evaporation and diffusion. It has been shown that the small variations in the isotopic composition of the water molecules in the snow that falls in Greenland are related to the temperature changes over the ice cap. In other words, if we are able to measure the water isotopic composition of the ice core from top to bottom we will be able to get an idea about the temperature history of Greenland from present times to tens of thousands of years in the past.

In isotope geochemistry, the isotopic composition of water is expressed with respect to an international standard water that is called Vienna Mean Ocean Water (VSMOW) and the symbol that is internationally used is the symbol $\delta$ ($\delta^{18}$O reflects differences in the number of oxygen atoms and $\delta^{2}$H reflects differences in the number of hydrogen atoms). As a rule, the more negative the value of $\delta^{18}$O is in the Greenland ice, the colder was the climate at the time when this piece of ice was deposited as snow on top of the Greenland ice sheet. Due to the fact that the quantity $\delta$ is a relative quantity, $\delta$ is unit-less and typically expressed in per mille.

You will be given two plastic bottles containing ice core sample in liquid form. The samples are taken from two different depths that are written on each bottle. Together with the bottles, you will be given a diagram presenting the water isotopic profile from the NEEM ice core (Figure 1.5) as well as the depth-age relationship for the top 1700 m of the NEEM ice core (Figure 1.6).

**Question 1.3.4**

Estimate the age of both samples and mark them clearly as vertical lines on Figure 1.5 and Figure 1.6. Then, using the isotopic composition plot you were given, estimate approximately the isotopic content $\delta^{18}$O you expect for each of the samples.

Which of the two samples do you think originates from a time when climate was significantly colder than now?

➢ *Give your answer in the answer sheet, box 1.3.4.*
Figure 1.5. $\delta^{18}\text{O}$ as a function of age (in years before year 2000) for the NEEM drilling site.

Figure 1.6. The depth-age relationship for top 1700 m of the NEEM ice core.
**Question 1.3.5**

Two different versions of a temperature – δ18O relationship are given by the equations below (T in °C and δ18O in per mille). The first one is linear and the second quadratic. Use both these equations to calculate the temperature above the ice cap at the time of deposition for the two samples you were given. What is the difference in temperature between the two samples?

\[ T = 1.5 \cdot \delta^{18}O + 20.45 \]

\[ T = -0.1 \cdot (\delta^{18}O)^2 - 4.46 \cdot \delta^{18}O - 72.43 \]

➤ *Give your answer in the answer sheet, box 1.3.5.*

**Sample preparation**

You will now proceed to the transfer of ice core sample to glass measurement vials. For this, you are given two pipettes, which you can use for this task. You need approximately 1.5 mL of water to be transferred from the sample bottle to the sample vial. Sample preparation is an important step in the process of measuring isotopic ratios and if not done properly it can lead to measurement errors.

**Keep in mind the following points when working:**

- Evaporation of the sample can alter its isotopic composition.
- Mixing of different waters with the sample will cause isotopic contamination.

**Question 1.3.6**

Proceed with the sample transfer. Label the sample vials carefully. Your samples will be analysed with a Cavity Ring Down Laser Spectrometer overnight in order to assess the quality of your sample preparation work.

➤ *Call a laboratory assistant to fetch and verify your sample.*

**Question 1.3.7**

Answer with True/False the four statements given below.

<table>
<thead>
<tr>
<th>Statement</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>The time that a sample is exposed to lab air can affect the quality of the measurement.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only one pipette should be used for the transfer of both samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keeping the samples as cold as possible helps minimizing isotopic fractionation.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The exact quantity of water transferred to the sample vials is critical for the quality of the isotopic analysis.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

➤ *Give your answers in the answer sheet, box 1.3.7.*
Experiment 2 32 marks

Copper and zinc content in an ice core

The volcano Laki in Iceland erupted on June 8, 1783 and the eruption lasted until February 1784. The amount of gases and ash emitted into the atmosphere affected the climate in Europe. Chemical analyses of ice cores, obtained from drillings in Greenland give valuable information of such incidents. The ice has been formed from pressurized snow of annual snowfalls. Each annual layer can give information about the temperature and composition of the atmosphere, including a possible content of volcanic ash in the year of the snowfall. Investigations have shown that volcanic ash contains metals.

In this experiment, an annual layer from an ice core is analysed for its content of the metals copper and zinc. By comparison with the results from other drillings, it can then be shown if the content of copper and zinc can be related to the eruption of Laki.

Materials and equipment

- Funnel
- Burette, 25 mL
- Erlenmeyer flask, 100 mL
- 3× Beaker, 250 mL
- 3× Graduated cylinder, 10 mL
- Pipettes, 5, 10 (3×), 15, 20 (2×), 25 (2×) mL
- Peleus ball
- 6× Volumetric flask, 100 mL
- Volumetric flask, 50 mL
- 10× Plastic cuvette, 10 mm
- Test tube, 20 mL
- 10× Plastic pipettes, 1 mL
- 50 mL 0.20 M CH₃COOH/1.7 M NaCH₃CO₂ buffer solution
- 10 mL 0.50 % Xylenol Orange indicator solution
- 50 mL 0.20 M Na₂S₂O₃ solution
- 250 mL 1.3 M NH₄Cl/7.0 M NH₃, pH = 10 buffer solution
- 100 mL 0.0360 M Cu(ClO₄)₂ solution
- 250 mL 0.0170 M EDTA solution
- 150 mL (Cu/Zn) analysis solution
- Vernier SpectroVis Plus Spectrophotometer
- Computer
- Plastic wash bottle
- Waste container labelled “X”
- Millimetre graph paper

For sample preparation, 180.6 g of ice core was melted and the liquid was quantitatively transferred to a 2000 mL volumetric flask. The flask was then filled up to the mark with water. The solution provided, labelled (Cu/Zn), is 10⁴ times more concentrated than the initial one.

In this experiment, the amount concentration of Cu²⁺ and Zn²⁺ in the analysis solution (Cu/Zn) has to be determined. The concentration of Zn²⁺ is determined by an EDTA-titration and the concentration of Cu²⁺ by spectrophotometry.

The content of Cu²⁺ and Zn²⁺ in the ice core can then finally be calculated.
A. EDTA (EthyleneDiamineTetraAcetate) titration

\[
\text{H}_4\text{edta}, \text{ commonly abbreviated EDTA, forms very stable complexes with metal ions } M^{q+} \text{ through the release of } H^+ \text{ ions:}
\]

\[
M^{q+} + H_4\text{edta} \rightleftharpoons M(\text{edta})^{(4-q)-} + 4 H^+
\]

This is why many metal ions in a not too acidic solution can be titrated with EDTA in a so-called complexometric titration.

The equivalence point of the titration is detected by using a metal ion indicator, in this case Xylenol Orange, which is bound to the metal ion with one colour (red) before the equivalence point and after the equivalence point exists as the free indicator with another colour (yellow) at the given pH. As titrator, a solution of Na\(_2\)H\(_2\)edta \(\cdot\) 2H\(_2\)O (hereafter referred to as EDTA-solution) is used.

Cu\(^{2+}\) is “masked” in the following EDTA-titration by reaction with excess of thiosulphate S\(_2\)O\(_3\)\(^{2-}\):

\[
2 \text{Cu}^{2+} + 6 \text{S}_2\text{O}_3^{2-} \rightarrow 2 \text{Cu} (\text{S}_2\text{O}_3)_2^{3-} + \text{S}_4\text{O}_6^{2-}
\]

The resulting complex Cu\((\text{S}_2\text{O}_3)_2^{3-}\) does not react with H\(_2\)edta\(^{2-}\). However, Zn\(^{2+}\) does not form a complex with thiosulphate, and as a result, it does react with H\(_2\)edta\(^{2-}\). In the following EDTA-titration, only the content of Zn\(^{2+}\) is determined:

\[
\text{Zn}^{2+} + \text{H}_2\text{edta}^{2-} \rightarrow \text{Zn(edta)}^{2-} + 2 \text{H}^+
\]
The burette provided has been filled with deionized water. The burette is first emptied, then rinsed and filled up with the 0.0170 M EDTA-solution. With a pipette, 10.00 mL of the analysis solution (Cu/Zn) is transferred to the 100 mL Erlenmeyer flask. Then, 5 mL of ethanoic acid/ethanoate buffer solution (0.20 M CH₃COOH/ 1.7 MNaCH₃CO₂) and 5 mL of 0.20 M Na₂S₂O₃ solution are added. To the colourless solution, 6 drops of 0.5 % Xylenol Orange solution are added and the solution is then titrated with the EDTA solution until the colour of the solution changes from red to lemon yellow.

**Question 2.1**
- The volume of titrant \( V_1 \) is written on the answer sheet, box 2.1.

The titrated mixture is poured into the waste container labelled X. The titration is repeated twice more and the values of \( V_1 \) are written on the answer sheet, box 2.1.

- Calculate the average value \( V_{1,av} \) and write the result on the answer sheet, box 2.1.

**Question 2.2**
In the analysis solution (Cu/Zn), calculate \([\text{Zn}^{2+}]\) from the value of \( V_{1,av} \).

- Show your calculation and write the result on the answer sheet, box 2.2.

**Question 2.3**
Why does \( \text{Cu(S}_2\text{O}_3\text{)}_2^{3-} \) not react with \( \text{H}_2\text{edta}^{2-} \)?

- Mark the correct answers on the answer sheet, box 2.3.

**Question 2.4**
Ethanoic acid, CH₃COOH, is a weak acid with the acid dissociation constant \( K_a = 1.78 \cdot 10^{-5} \) M, where \( K_a \) can be expressed as:

\[
K_a = \frac{[\text{H}_3\text{O}^+][\text{CH}_3\text{CO}_2^-]}{[\text{CH}_3\text{COOH}]}
\]

- Isolate \([\text{H}_3\text{O}^+]\) in the equation and write the expression on the answer sheet, box 2.4.

Calculate \([\text{H}_3\text{O}^+]\) in the ethanoic acid/ethanoate buffer solution.

- Show the result on the answer sheet, box 2.4.

Calculate pH in the buffer solution.

- Write the expression and the result on the answer sheet, box 2.4.

For a solution containing a corresponding acid-base pair the mole fraction of acid, \( X_a \), gives the fraction of the corresponding acid-base pair present in the acidic form. The mole fraction of acid, \( X_a \), depends on pH in the solution. This correlation between pH and \( X_a \) for a corresponding acid-base pair can be expressed graphically in a so-called Bjerrum plot.
H$_4$edta is a tetravalent acid with the $pK_a$ values $pK_{a1} = 1.99$, $pK_{a2} = 2.67$, $pK_{a3} = 6.16$, $pK_{a4} = 10.22$. From these values, the Bjerrum plot for the EDTA-system can be constructed:

![Bjerrum plot for the EDTA-system.](image)

**Question 2.5**

Using the Bjerrum plot, *estimate* whether an aqueous solution of Na$_2$H$_2$edta $\cdot$ 2H$_2$O is acidic, basic or neutral. Justify the answer by a marking on the pH axis in the Bjerrum plot.

➤ *Write the answer on the answer sheet, box 2.5.*

**B. Spectrophotometry**

Coloured compounds absorb light in the visible range ($\lambda = 400 - 700$ nm). In a cuvette containing a solution of a compound $S$, the absorbance ($A$) depends on the path length ($l$), the concentration of $S$ ($[S]$), and the wavelength dependent molar absorption coefficient ($\varepsilon$) of $S$ in the following way:

$$A = \varepsilon \cdot [S] \cdot l$$

This equation is called the Beer-Lambert Law.

The metal ions Cu$^{2+}$ and Zn$^{2+}$ react quantitatively with ammonia in aqueous solution forming the compounds Cu(NH$_3$)$_4^{2+}$ and Zn(NH$_3$)$_4^{2+}$:

$$M^{2+} + 4 \text{NH}_3 \rightarrow M(\text{NH}_3)_4^{2+} \quad (M^{2+} = \text{Cu}^{2+}, \text{Zn}^{2+})$$

At a given wavelength, the absorbance of such a solution is equal to the sum of contributions from all compounds in the solution:

$$A = A(\text{Cu(NH}_3)_4^{2+}) + A(\text{Zn(NH}_3)_4^{2+})$$

This can be rewritten as:

$$A = \varepsilon(\text{Cu(NH}_3)_4^{2+}) \cdot [\text{Cu(NH}_3)_4^{2+}] \cdot l + \varepsilon(\text{Zn(NH}_3)_4^{2+}) \cdot [\text{Zn(NH}_3)_4^{2+}] \cdot l$$
In the entire visible range $\varepsilon(Zn(NH_{3})_{4}^{2+}) = 0 \text{ M}^{-1}\text{cm}^{-1}$ and the absorbance of an aqueous solution containing $Cu^{2+}, Zn^{2+}$ and excess of $NH_{3}$ is therefore:

$$A = \varepsilon(Cu(NH_{3})_{4}^{2+}) \cdot [Cu(NH_{3})_{4}^{2+}] \cdot l$$

The absorbance is determined using a Vernier SpectroVis Plus Spectrophotometer connected to a computer and cuvettes with a path length of $l = 1.00 \text{ cm}$ are used. In the following an ammonium/ammonia buffer solution ($1.3 \text{ M NH}_{4}\text{Cl} / 7 \text{ M NH}_{3}, \text{ pH} = 10$) is used as a source of ammonia and a 0.0360 M solution of copper(II) perchlorate, $Cu(ClO_{4})_{2}$, as a source of $Cu^{2+}$.

Six solutions (1-6) with known concentrations of $Cu(NH_{3})_{4}^{2+}$ are prepared by transferring aliquots of 0.0360 M $Cu(ClO_{4})_{2}$ to six separate 100 mL volumetric flasks using pipette volumes 0, 5.00, 10.00, 15.00, 20.00, and 25.00 mL, respectively. To each flask is then added 20.00 mL ammonium/ammonia buffer solution ($1.3 \text{ M NH}_{4}\text{Cl} / 7 \text{ M NH}_{3}, \text{ pH} = 10$). The flasks are filled to the mark with water and shaken thoroughly.

**Question 2.6**

For each of the solutions 2-6 calculate the concentration of $Cu(NH_{3})_{4}^{2+}$.

➢ *Write the answers on the answer sheet, box 2.6.*

Using solution 1 as a blank, measure the absorbance at $\lambda = 618 \text{ nm} (A_{618})$ of solutions 2-6. (Appendix C gives instructions for use of the spectrophotometer.)

➢ *Write the results on the answer sheet, box 2.6.*

Using a pipette, 25.00 mL of the analysis solution ($Cu/Zn$) is transferred to a 50 mL volumetric flask. Then 10.00 mL ammonium/ammonia-buffer solution ($1.3 \text{ M NH}_{4}\text{Cl} / 7 \text{ M NH}_{3}, \text{ pH} = 10$) is added using a pipette. The flask is filled to the mark with water and shaken thoroughly. This solution is labelled 7.

**Question 2.7**

**Measure** the absorbance at $\lambda = 618 \text{ nm} (A_{618})$ of solution 7.

➢ *Write the result on the answer sheet, box 2.7.*

Plot the values of $A_{618}$ vs. $[Cu(NH_{3})_{4}^{2+}]$ for solutions 2-6 on the supplied graph paper (label the graph “Graph 2.7”). Draw a line that best fits the points and calculate the slope and $y$-intercept.

➢ *Add Graph 2.7 to the answer sheet.*

**Question 2.8**

Find the slope and $y$-intercept.

➢ *Show your readings on the graph paper, and show your calculations and results on the answer sheet, box 2.8.*
Question 2.9

Calculate the molar absorption coefficient, $\varepsilon$, for $\text{Cu(NH}_3\text{)}_4^{2+}$ at $\lambda = 618$ nm.

➢ Write the calculation and result on the answer sheet, box 2.9.

Question 2.10

Using the $A_{618}$-value of solution 7 calculate $[\text{Cu}^{2+}]$ in the analysis solution (Cu/Zn).

➢ Write the calculation and result on the answer sheet, box 2.10.

Using a graduated cylinder, transfer 10 mL of the analysis solution (Cu/Zn) to a test tube. Add the ammonium/ammonia buffer solution (1.3 M NH$_4$Cl/ 7 M NH$_3$, pH = 10) dropwise. After the addition of a few drops of the buffer solution a precipitate is formed. Add more buffer solution dropwise with stirring until the precipitate is completely redissolved.

Question 2.11

Suggest, with a chemical formula, what the identity of the precipitate could be.

➢ Write the answer on the answer sheet, box 2.11.

➢ Write a balanced reaction scheme for the formation of the precipitate on the answer sheet, box 2.11.

C. The ice core

Question 2.12

Calculate the content of Cu$^{2+}$ and Zn$^{2+}$ in pg/g in the ice core (1 pg = $10^{-12}$ g). Molar masses: Cu: 63.54 g/mol; Zn: 65.38 g/mol.

➢ Write the calculations and results on the answer sheet, box 2.12.

In another drilling from Greenland, eight ice cores from depths of 67.155 – 67.785 m containing annual layers from the time period 1782–1785 have been analysed for the content of copper and zinc with the following results:

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Sample number</th>
<th>Cu (pg/g)</th>
<th>Zn (pg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.155–67.23</td>
<td>1</td>
<td>0.46</td>
<td>35</td>
</tr>
<tr>
<td>67.23–67.305</td>
<td>2</td>
<td>1.6</td>
<td>26</td>
</tr>
<tr>
<td>67.315–67.40</td>
<td>3</td>
<td>2.9</td>
<td>42</td>
</tr>
<tr>
<td>67.485–67.485</td>
<td>4</td>
<td>6.4</td>
<td>35</td>
</tr>
<tr>
<td>67.485–67.555</td>
<td>5</td>
<td>2.0</td>
<td>11</td>
</tr>
<tr>
<td>67.555–67.625</td>
<td>6</td>
<td>20.5</td>
<td>490</td>
</tr>
<tr>
<td>67.645–67.745</td>
<td>7</td>
<td>3.2</td>
<td>21</td>
</tr>
<tr>
<td>67.745–67.785</td>
<td>8</td>
<td>1.2</td>
<td>24</td>
</tr>
</tbody>
</table>
It was concluded that the relatively high content of copper and zinc in sample number 6 could be related to the eruption of Laki. By comparison with your results, estimate if the content of copper and zinc in your ice core can be related to the eruption of Laki.

Mark the correct answer on the answer sheet, box 2.13.
Experiment 3

Metazoan life in extreme environments

Many different types of extreme and harsh habitats exist around the globe. In such habitats, living organisms are challenged by extremes in physicochemical factors, such as temperature, water availability, salinity, pH, and oxygen tension. In order to cope with these hostile environments, organisms require special adaptations and only the most resistant survive. Organisms that live in the Arctic are, obviously, adapted to very low temperatures. Cryoconite holes (created when dust melts through the snow or ice) offer a niche for selected algae, bacteria and metazoans. In this exercise, you are provided with a sample that mimics the material found in a cryoconite hole.

Figure 3.1. Cryoconite hole.

Materials

- Petri dish with sediment sample
- Stereo microscope
- Dissecting needle
- Identification Key (Appendix A1)
- Image Key (Appendix A2)

Find and identify metazoan lifeforms

Question 3.1

Find and identify active and moving microscopic metazoan lifeforms (up to 1 mm long) in the sediment sample using the stereo microscope and the “Identification Key” (Appendix A1).

➢ Fill in the answers in the answer sheet.
Note that:

1. Images (Appendix A2) are provided of animals that are present in the sample AND of animals that are NOT microscopic and/or NOT present in the sediment sample. Numbers marked with an asterisk (*) in the "Identification Key" (Appendix A1) refer to a specific image in Appendix A2.

2. You should consult the "Terminology" given below before filling in the answers.

3. Filling in the answer sheet correctly - i.e. with exactly the microscopic metazoans that are actually present in the sample will result in full points.

Terminology

Metazoan  A multicellular animal with cells differentiated into tissues.

Radial symmetry  The animal has a central axis along which it can be divided into a number of mirror images. The animal has no left and right side.

Appendages  External protuberances from the animal’s body, e.g. legs or antennae.

Cilium (plural cilia)  Slender, hair like process extending from the surface of a cell. Cilia may be motile or non-motile.

Scalids  Spinose appendages that function in locomotion, chemo- and mechano-reception.

Articulated  Consisting of sections united by joints.
Ancient Greenlandic habitats

Deep below the ice cap all the way down at bedrock, remnants of life that existed before the ice covered the landscape can be found. Samples have been taken 2-3000 meter down in the ice (See Figure 4.1) from the ice core drilling site Dye-3. By looking at the basal ice, which contains soil particles, it is possible to find soil material that has been scraped up by the movement of the ice. Hence, the basal ice may contain a lot of ancient genetic material, which can give us an indication of the past climate and plant diversity at the time before ice covered Greenland. Ancient soil DNA has been extracted from this material.

In this experiment, you will analyse DNA from such samples and determine what kind of habitat existed in Greenland before the ice was formed. You will look for the presence of indicator plants, which thrive in certain climate niches and have specific temperature requirements for survival and growth. The different growth conditions required for the specific plant families can tell us something about the temperature ranges of their habitat, length of summer and winter period and if for example forest existed. From this kind of information, you will be able to explain what kind of habitat you would expect to see in Greenland before the great ice cap was formed.

A PCR (Polymerase Chain Reaction) has been performed to amplify the DNA we would like to analyse. Your job will be to analyse the ancient environmental DNA by gel-electrophoresis and you will compare these results to DNA libraries of modern day plants.
To see the presence of a specific plant family, primers have been designed to target a specific region in the genome for each indicator family of interest. If an indicator family is present in your sample, you will see a band on your gel.

Materials

- 1 gel cassette
- 1 FlashGel® Dock with cables and power supply
- 1 Power source
- 1 tube of buffer, labelled “Buffer 4”
- 1 micropipette 2-20 µL
- Micropipette tips
- PCR-tubes containing DNA samples with primers for indicator families (for Experiment 4A)
- PCR-tubes containing DNA samples with primers for indicator genera (for Experiment 4B)
- Lint free wipes
- Disposal bag for waste
- Appendix B

Taxonomic tree

You will be looking for specific plant families and genera in your samples. These families and genera are used as climate indicators, since these can only grow if the climate fits their needs. When analysing your results, you will need Appendix B, Figure 4.2 and Figure 4.3.

---

1 Primers are short single stranded DNA fragments (20-30 nucleotides long) that are designed to bind to a complementary DNA strand. During a PCR, the DNA polymerase will start copying the complementary DNA strand from the positions of the primer. Hence, the complementary DNA strand will be amplified.
Electrophoresis

You will be using the electrophoresis technique as part of your investigation on the ancient DNA fragments in the ice.

Question 4.1

➢ Write the letters for the correct words in the answer sheet, box 4.1.

Words

<table>
<thead>
<tr>
<th>A Amino acid</th>
<th>G Base</th>
<th>M Wells</th>
<th>S Deoxyribose-molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>B DNA-ladder</td>
<td>H DNA-fragments</td>
<td>N Fatty acids</td>
<td>T Phosphate-groups</td>
</tr>
<tr>
<td>C Glucose molecules</td>
<td>I Ditches</td>
<td>O Shortest</td>
<td>U Carbohydrates</td>
</tr>
<tr>
<td>D Charge</td>
<td>J Length</td>
<td>P Longest</td>
<td>V Loading dye</td>
</tr>
<tr>
<td>E Molecules</td>
<td>K Negative pole</td>
<td>Q Net negative charge</td>
<td>W Positive pole</td>
</tr>
<tr>
<td>F Net positive charge</td>
<td>L Proteins</td>
<td>R Size</td>
<td>X Macromolecules</td>
</tr>
</tbody>
</table>

Text

An electrophoresis is conducted in an electrophoresis cassette. Samples are loaded into the {___ Word 1 ___} of a gel, which is covered with buffer.

When placed in an electric field, macromolecules such as {___ Word 2 ___} and {___ Word 3 ___} can be separated. The separation depends on the different {___ Word 4 ___} and {___ Word 5 ___} of the molecules.

Nucleic acids migrate towards the {___ Word 6 ___} as they have a {___ Word 7 ___} due to the {___ Word 8 ___}. Proteins, however, could migrate towards either the positive or the negative pole, since their overall charge depends on their {___ Word 9 ___} composition.
Experiment 4A. Analyse the DNA samples using gel-electrophoresis

Ancient DNA bound to soil particles preserved in the basal ice under the ice cap is a glimpse into ancient natural history. Since the material has been frozen and kept oxygen free, nucleotide sequences have been preserved, however, in a highly degraded state. We can use the remaining short degraded pieces of DNA code as a taxonomic library of the previous genera from this geographical area of Greenland. See the list below of the different families we are looking for, in the samples obtained by PCR-amplification using family-specific primers:

A1. Taxaceae
A2. Pinaceae
A3. Betulaceae
A4. Fabaceae
A5. Fagaceae

The different DNA samples indicate different indicator families that we are looking for.

Instructions

1. Tear open pouch and remove cassette.
2. Remove seals from cassette.
3. Make sure sample wells become flooded with “Buffer 4” and remove buffer from cassette, but do not remove buffer from the wells. If in doubt, please ask the instructor – there will be no penalty for asking!
4. Insert cassette into dock.
5. Load samples from PCR tubes (5 µL pr. sample) as shown in the Figure 4.4 below. Remember to write down which samples are loaded where.

**NB: Use only 5 wells, as another 6 wells will be needed later on.**

6. Plug in cables as indicated by colour code to the power source.
7. Set power to: Voltage 195 V DC or as posted at lab table, Power 15 W or as posted, Current: 25 mA, and time to 10 min.

8. Start the run on the power source.

9. Observe the experiment every minute by turning on the light on the light switch. Let the experiment run for maximum 9 minutes.

![Application of a sample on to the gel.](image)

**Figure 4.4. Application of a sample on to the gel.**

**Analysing the results of experiment 4A**

**Question 4.2**

If you obtain a signal on the gel, the family was present. Write the names of the families you have analysed in the gel boxes in the answer sheet and indicate if the family was present or not present in your sample. This is done by writing + (present) or – (not present) on the gel template in the answer sheet under the name of the family.

➢ **Write the names of the families you have analysed on the answer sheet, box 4.2.**

**Question 4.3**

Based on the data from experiment A and the information in Appendix B, decide whether the following statements about Greenlandic flora are true or false.

<table>
<thead>
<tr>
<th>Statements</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plants were present at the time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only four families existed at the time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The winters were below –2 °C and the summers were above 10 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only three families existed at that time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing can be concluded about the temperature by information based on only the families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greenland had a forest at the time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

➢ **Tick the answers in box 4.3 on the answer sheet.**

**Question 4.4**

Which families would be relevant to investigate for further analysis? Taxaceae, Fagaceae, Pinaceae, Fabaceae and/or Betulaceae?
Experiment 4B. Analyse DNA samples using gel-electrophoresis

The DNA samples indicated which indicator genera we should be looking for.

See the list below of the different genera we are looking for, in the DNA-samples obtained by PCR-amplification using genera-specific primers:

B1. Alnus  
B2. Picea  
B3. Pinus  
B4. Taxus  
B5. Cassia  
B6. Castanea

Instructions

1. Make sure sample wells become flooded with buffer and remove buffer 4 from cassette, but do not remove buffer in the wells.

2. Insert cassette into dock.

**NB: Be aware to use the wells NOT used in the previous experiment!**

3. Load samples from PCR tubes (5 µL pr. sample). Remember to write down which samples are loaded where.

4. Plug in cables as indicated by colour code to the power source.

5. Set power to: Voltage 195 V DC or as posted at lab table, Power 15 W or as posted, Current: 25 mA, and time to 10 min.

6. Start the run on the power source.

7. Observe the experiment every minute by turning on the light on the light switch. Let the experiment run for maximum 9 minutes.

Analysing the results from experiment 4B

As in experiment 4A use the attached taxonomic tree and the table showing the indicator genera.

**Question 4.5**

Write the names of the genera you have analysed in the boxes and indicate if the genus was present or not present in your sample.

➢ *This is done by marking either + (present) or – (not present), on the answer sheet, box 4.5.*
Question 4.6
Based on experiment 4B, Appendix B, and the above answers, what kind of ecosystem was dominant at the location of Dye-3?

<table>
<thead>
<tr>
<th>Statement</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainforest.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deciduous temperate forest.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mire (a wetland terrain without forest cover, dominated by living, peat-forming plants).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meadow (an open area with grassland).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boreal forest with a mix of conifers and deciduous trees.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

➢ Tick the correct answer on the answer sheet, box 4.6.

Question 4.7
We want to be sure that the DNA from the basal ice samples are really representing the ancient ecosystems and not just contaminations from the air that was transported to Greenland from other areas through time. Where would you take control samples in the ice core (see Figure 4.1) to check for airborne exotic DNA?

<table>
<thead>
<tr>
<th>Statement</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the centre of the glacial ice core and close to the basal ice where exotic plant DNA might have been incorporated together with air, airborne contaminants and snow.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the clean glacial ice much closer to the surface than to the basal ice.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only on top of the ice cap since this place is most likely to be contaminated.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atmospheric air samples since this is where the contaminants are.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atmospheric air samples and top of the ice cap since both contain the contaminants.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

➢ Tick your answers on the answer sheet, box 4.7.

Question 4.8
From the indicator genera, make an analysis on what the climate most likely looked like at the time these organisms were living in Greenland – what are the upper and lower temperature boundaries? Use Appendix B.

<table>
<thead>
<tr>
<th>Statement</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summers are more than 10 °C warm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winters are down to −40 °C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winters are not colder than −17 °C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winters does not go below −1 °C.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Backtracking the protein sequence

The researchers at Dye-3 found a fraction of a protein when studying their findings. Your job is to backtrack it to mRNA and choose a possible specific primer for testing it using PCR (polymerase chain reaction).

The protein sequence is:

Met-Phe-Asp-Gln-Asp-Tyr-Trp

**Question 4.9**

Using the genetic code (Figure 4.5), calculate the possible number of mRNA combinations of the protein sequence.

<table>
<thead>
<tr>
<th>Second Letter</th>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>UUU UUC UUA UUG</td>
<td>Phe</td>
<td>Ser</td>
<td>UAU UAC UAA UAG Stop</td>
</tr>
<tr>
<td>C</td>
<td>CUU CUC CUA CUG</td>
<td>Leu</td>
<td>Pro</td>
<td>CAU CAC CAU CAG His Gln</td>
</tr>
<tr>
<td>A</td>
<td>AUG AUG AUG AUG</td>
<td>Met</td>
<td>Thr</td>
<td>Asn Asn Asn Asn Lys</td>
</tr>
<tr>
<td>G</td>
<td>GUU GUC GUA GUG</td>
<td>Val</td>
<td>Ala</td>
<td>Asp Asp Asp Asp Glu</td>
</tr>
</tbody>
</table>

**Figure 4.5.**

- Write your answer on the answer sheet, box 4.9.

**Question 4.10**

Which of these mRNA sequences is one of the possible combinations of the protein sequence?

- 5’ - AUG UUU GAU GAG GAC UAU UGG - 3’
- 5’ - AUG UUC CCA CAG GAC UAC UGG - 3’
- 5’ - AUG UUC GAU CAG GAC UAC UGG - 3’
- 5’ - AUG UUU GAU GGA GAU UAU UGG - 3’
- 5’ - AUG GGA GAU CAG GAU UAU UGG - 3’

- Write your answer on the answer sheet, box 4.10.
**Question 4.11**

You are only asked to choose one primer 12 bp long, although this is far too short to be specific, when it comes to real DNA-analysing. Normally you will use both a forward and a reverse primer.

Which of these would you use as a specific primer for further analysis?

\[
\begin{align*}
3' & -CTC\ CTG\ ATA\ ACC-5' \\
3' & -GTT\ CTG\ ATG\ ACC-5' \\
3' & -GTC\ CTG\ ATG\ ACC-5' \\
3' & -CTT\ CTA\ ATA\ ACC-5' \\
3' & -GTC\ CTA\ TTA\ ACC-5'
\end{align*}
\]

➢ Write your answer on the answer sheet, box 4.11.

Now when we know some of the plants and temperature conditions of Greenland before the great ice cap formed and covered most of the land area, we want to know when this ice build-up began in the area of the Dye-3 core.

The basal ice has been dated by a combination of four dating techniques; see below. Two of the methods are based on physics and two are based on biology, and the basic theory behind them will be described below.

1. The first physics method to date the ice is based on isotope decay of radioactive isotopes in the ice, like \(^{10}\text{Be}\) and \(^{36}\text{Cl}\). These two isotopes are present in the atmosphere and get incorporated into the ice cap together with the snow. This method estimates the ratio of decay of the \(^{10}\text{Be} / {^{36}\text{Cl}}\) isotopes, which is occurring exponentially with time. Therefore, this method will provide an age estimate based on how long the isotopes and the air have been incorporated in the ice. This is theoretically equal to the age of the ice.

2. Another physics method is optical stimulated luminescence (OSL) dating. This method estimates the time since the soil particles last received any daylight, and hence the time since they got incorporated into the ice. By applying a strong laser beam on the soil particles one can estimate the amount of light reflected back from particles of the soil minerals: feldspar or quartz. The amount of light reflected back is proportional to the last time the particles were exposed to light. This is also theoretically giving us an estimate of when the ice formed and thereby its age.

3. The first biological method applied is called amino acid racemization (AAR). This is measuring the decay of amino acids from biological tissue in organisms. The decay rate in certain amino acids is constant in cold temperatures. Therefore, we can theoretically estimate the age of the ice based on the level of decay of these amino acids within the basal ice samples.

4. The second biological method is based on DNA and the molecular clock theory. Since DNA molecules degrade with time and obtain certain erroneous mutations at a certain rate, we can compare the ancient DNA sequences with modern ones in specific gene regions. Thereby we can get an estimate of the age of the ancient DNA sequences. This will indicate when the organism lived and hence when the ice started to build up and incorporated the DNA sequences.
All four dating techniques have been developed fairly recently to be applied on samples of basal ice, since no other well-tested methods have been available for this kind of material. Each of these four methods are therefore prone to provide some level of uncertainties. It was therefore decided to combine the four different methods to get a consensus age from all of them.

**Figure 4.6.** Four dating methods used to determine the age of the ancient DNA in the ice cap. Horizontal timeline: Years before present x 1000. **LIG:** Last interglacial period, approx. 120,000 ybp. Vertical axis: The four methods. Dashed lines indicate uncertainty of the maximum or minimum age.

**Question 4.12**

By using **Figure 4.6**, can you determine the last time that forest occurred in the Dye-3 location from a combination of the four methods?

➤ **Tick the answers on the answer sheet, box 4.12.**

END OF TASK 1: ICE