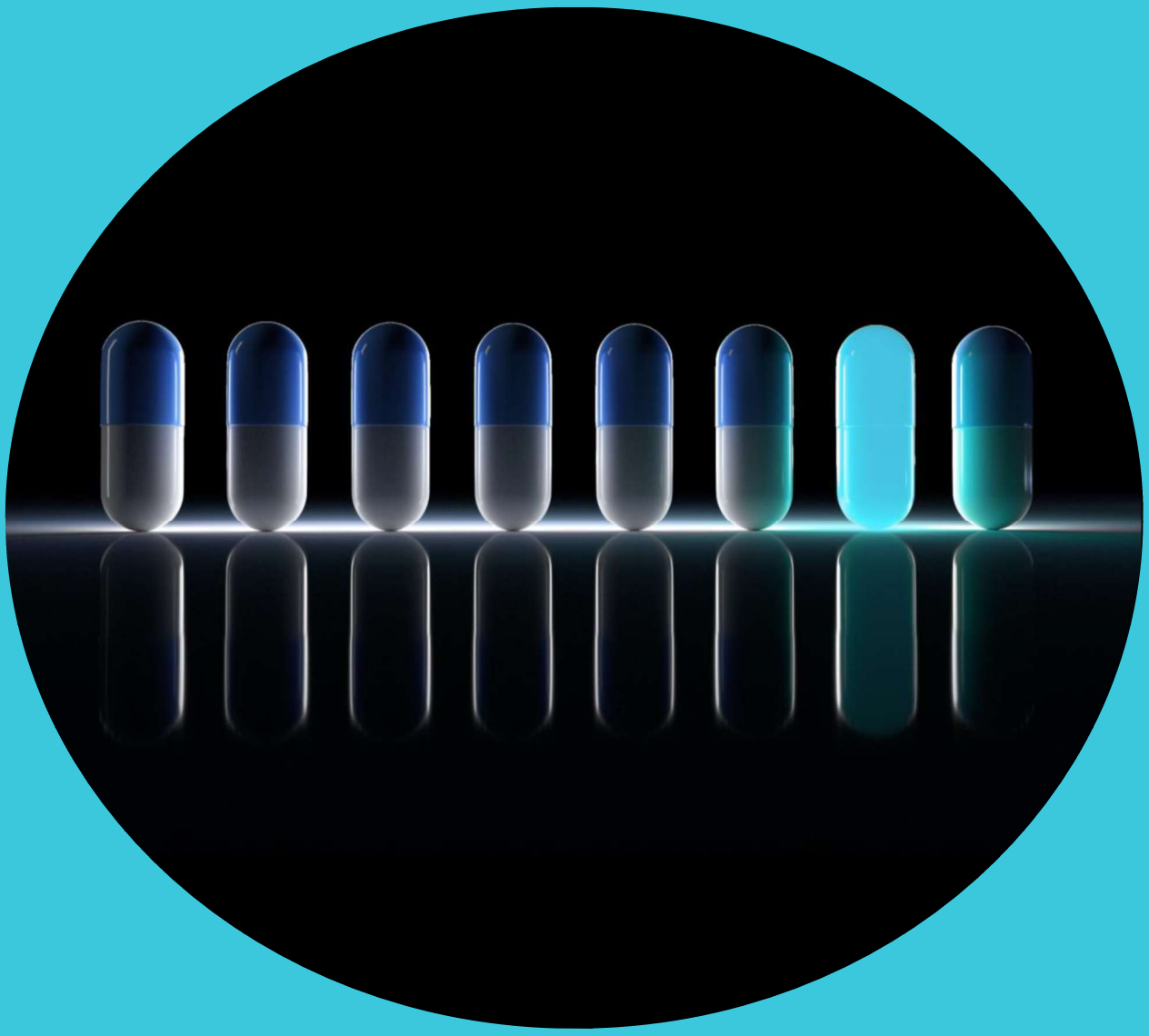
Researchers in Schools



Glow in the Dark Drugs

Key Stage 4 Programme

Pupil Name

Coursebook Designed by **Dr Thomas D.Z. Pearson**

Timetable and Assignment Submission

Timetable – Tutorials

Tutorial	Date	Time	Location
1	2nd December 2019	9am – 4pm	Senate House Library University of London
2	9th January 2020	3pm – 4pm	Lord's Hill
3	16th January 2020	3pm – 4pm	Lord's Hill
4	23rd January 2020	3pm – 4pm	Lord's Hill
5	30th January 2020	3pm – 4pm	Lord's Hill
6 (Feedback)	13th February 2020	3pm – 4pm	Lord's Hill
7 (Feedback)	27th February 2020	3pm – 4pm	Lord's Hill
Graduation	19th March 2020	TBC	Lord's Hill

Timetable – Homework Assignments

Homework Assignment	Description	Due Date
Tutorial 1	Baseline Assignment	13th December 2019
Tutorial 2	Couple the Compounds	15th January 2020
Tutorial 3	Reperfusion Recall Poster	22nd January 2020
Tutorial 4	Crushing Numbers	29th January 2020
Tutorial 5	Final Assignment	11th February 2020

Assignment Submission – Lateness and Plagiarism

Lateness	
Submission after midnight on 9th August	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

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Course Rationale

Medicines are all around us and make up a part of people's daily life. It is rare that you or someone close to you will go through life having never taken a pill, had an injection, or spent time in surgery. The question is where do our medicines come from? Do we just find them growing on trees, or are they made in advanced laboratories? Can I make a medicine for myself? What is the medicine doing inside of me?

This course aims to explain the connections between chemistry and biology by studying how medicines are found and made, in order to understand the world around us. Using the University format of learning, the course will prepare the student for a higher level of study, giving an advantage in GCSE and A-level exams.

We will undertake a brief look at a subcellular organelle and its intimate relationship with heart disease. In the following tutorial we study the lock and key model for enzymes and see how that applies to chemicals and proteins. Some of the simple rules for making drugs will be explored, a practical experiment in drug design will be attempted, and a final lesson on the different uses of light to see the chemical binding of proteins, will provide the foundations for a student to understand drug development.

Uni Pathways Mark Scheme 2019 – Essay-based assignments

	Skills	1st (70-100)	2:1 (60-69)	2:2 (50-59)	3rd (40-49)	Mark /100
Subject knowledge	Knowledge and understanding	○ All content included and materials used are relevant to the general topic and to the specific question/title ○ Good understanding of all the relevant topics. ○ Technical terms are defined and used accurately throughout ○ Clear justification of how the material and content included is related to the specific issues that are the focus of the assignment	○ Most of the materials used and content included are relevant to the general topic and to the specific question/title ○ Good understanding of most the relevant topics ○ Technical terms are mostly defined and used accurately ○ Adequate justification of how the material used and content included are related to the specific issues that are the focus of the essay	○ Some of the materials used and content included are relevant to the general topic and to the specific question/title ○ Good understanding on some of the relevant topics but occasional confusion on others ○ Technical terms are sometimes used and defined accurately ○ Some justification of how the material used and content included are related to the specific issues that are the focus of the essay	○ The content included and materials used are not applied to the question/title in a relevant manner ○ There is confusion in how understanding of the topics is expressed	
Critical thinking	Research and evidence	○ Includes rich sources of research findings, data, quotations or other sourced material as evidence for the claims/ideas ○ Uses evidence/calculations to support claims/assertions/ideas, consistently clearly and convincingly ○ Evidence of further reading beyond materials provided which were used in an appropriate context ○ Data is effectively analysed and appropriate assumptions/conclusions are reached	○ Includes adequate sources of research findings, data, quotations or other sourced material as evidence for the claims/ideas ○ Uses evidence/calculations to support claims/assertions/ideas, mostly clearly and convincingly ○ Evidence of further reading beyond materials provided ○ Data is analysed and the assumptions/conclusions that are reached are mostly appropriate	○ Includes some sources of research findings, data, quotations or other sourced material as evidence for the claims/ ideas ○ Uses evidence/calculations to support claims/assertions/ideas, at times clearly and convincingly ○ Limited evidence of further reading beyond materials provided ○ There is an attempt to analyse data is and draw assumptions/conclusions	○ Inclusion sources and materials is very limited and mostly not attributed ○ Applicable sources are rarely used to support ideas. ○ Data is not used or few appropriate conclusions are drawn from it.	
	Developing an argument	○ A point of view or position in relation to the title or question is consistently clear. The position is developed effectively and consistently throughout the essay ○ Argument is exceptionally well-developed and well-justified ○ Makes links effectively between subjects that have not previously been associated ○ Uses concepts from the tutorials in an unfamiliar context and does so accurately and confidently. ○ Content is analysed effectively to support the argument	○ A point of view or position in relation to the title or question is adequately clear. The position is well-developed in most of the essay ○ Argument is clear and well-developed, and position is justified ○ Some evidence of linking subjects that have not previously been associated ○ Uses some concepts from the tutorials in an unfamiliar context, but not always accurately ○ Analyses content to support the argument	○ A point of view or position in relation to the title or question is somewhat clear. The position is well-developed in parts of the essay ○ Argument is clear but not well-developed ○ Limited evidence of linking subjects that have not previously been associated ○ Limited use of concepts from the tutorials in other contexts ○ Uses some analysis of content to support the argument	○ There is not a clear point of view or position taken and sometimes the argument is not clearly established	

	Critical evaluation	○ Moves beyond description to an assessment of the value or significance of what is described ○ Evaluative points are consistently explicit/systematic/reasoned/justified ○ Effectively critiques the reliability of sources provided	○ Mostly description but some assessment of the value or significance of what is described ○ Evaluative points are mostly explicit/systematic/reasoned/justified ○ Some evidence of critiques on the reliability of sources provided	○ Describes with minimal assessment of the value or significance of what is described ○ Evaluative points are at times explicit/systematic/reasoned/justified ○ Limited evidence of critiques on the reliability of sources provided	○ The work is descriptive in nature and there is a lack of critical engagement in the value of sources	
Written communication	Structure and presentation	○ Ideas are presented in paragraphs and arranged in a logical structure that is appropriate for the assignment ○ The introduction clearly outlines how the essay/report will deal with the issues ○ The conclusion summarises all the main points clearly and concisely ○ Tables and graphs are effectively constructed including appropriate headings, units and scales. ○ All sources are referenced correctly in an agreed format	○ Ideas are presented in paragraphs and arranged in a structure that is mostly appropriate for the assignment ○ The introduction adequately describes how the essay/report will deal with the issues ○ The conclusion summarises most of the main points clearly ○ Most tables and graphs are well constructed ○ Most sources are referenced correctly in an agreed format	○ Ideas are presented in paragraphs and arranged in a structure ○ The introduction mentions how the essay/report will deal with the issues ○ The conclusion summarises some of the main points clearly ○ Some tables and graphs are well constructed but contains some errors ○ Some sources are referenced correctly in the agreed format with occasional errors	○ Ideas are presented in paragraphs but there is a lack of structure in how the work is presented ○ The work lacks an introduction that establishes the scope of the question ○ The work lacks a conclusion that summarise the main points raised ○ Work is not referenced accurately	
	Language and style	○ No spelling, grammar or punctuation errors ○ Writing style consistently clear, tone appropriate and easy to follow ○ Accurate and consistent use of technical language and vocabulary	○ Minimal spelling, grammar or punctuation errors ○ Writing style mostly clear, tone appropriate and easy to follow ○ Some attempts of using technical language and vocab alary, but not always accurate	○ Some spelling, grammar or punctuation errors ○ Writing style moderately clear, tone appropriate and easy to follow ○ Use of simple language and vocabulary effectively but struggles to use technical language	○ There are a significant number of spelling, grammar and punctuation errors ○ Use of simple language and vocabulary effectively but a lack of technical language	
Overall Mark (average of the 6 marks from the criteria above)						

Baseline Assignment (essay based): Pupil Feedback Report

Name of Pupil	
Name of School	Oasis Academy Lord's Hill
Name of RIS teacher	Dr T. Pearson
Title of Assignment	Drug Literature Review

How your assignment is graded:

Grade	Marks	What this means
1st	70+	Performing to an excellent standard at A-level
2:1	60-69	Performing to a good standard at A-level
2:2	50-59	Performing to an excellent standard at GCSE
3rd	40-49	Performing to a good standard at GCSE
Working towards a pass	0-39	Performing below a good standard at GCSE
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Marks

OVERALL MARK / 100		FINAL MARK / 100 (including any deductions)	
DEDUCTED MARKS		FINAL GRADE	

If marks have been deducted (e.g. late submission, plagiarism) the teacher should give an explanation in this section:

Mark Breakdown and Feedback

Knowledge and Understanding		Research and Evidence	
	mark		*mark*
Developing an Argument		**Critical Evaluation**	
	mark		*mark*
Structure and Presentation		**Language and Style**	
	mark		*mark*

Final Assignment (essay based): Pupil Feedback Report

Name of Pupil	
Name of School	Oasis Academy Lord's Hill
Name of RIS teacher	Dr T. Pearson
Title of Assignment	Drug Development Brief

How your assignment is graded:

Grade	Marks	What this means
1st	70+	Performing to an excellent standard at A-level
2:1	60-69	Performing to a good standard at A-level
2:2	50-59	Performing to an excellent standard at GCSE
3rd	40-49	Performing to a good standard at GCSE
Working towards a pass	0-39	Performing below a good standard at GCSE
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Marks

OVERALL MARK / 100		FINAL MARK / 100 (including any deductions)	
DEDUCTED MARKS		FINAL GRADE	

If marks have been deducted (e.g. late submission, plagiarism) the teacher should give an explanation in this section:

Mark Breakdown and Feedback

Knowledge and Understanding		Research and Evidence	
	mark		*mark*
Developing an Argument		**Critical Evaluation**	
	mark		*mark*
Structure and Presentation		**Language and Style**	
	mark		*mark*

Subject Vocabulary

Word	Definition	In a sentence
Activity Assay	The testing of how active a biological protein or enzyme is.	We can discover how well proteases that are found in the stomach can work, in an activity assay.
Cardiac	Relating to the heart.	The heart is made from cardiac tissue.
Efficacy	How well something works.	How good is paracetamol's efficacy?
Electrophoresis	Movement of particles in a fluid when an electric current is passed through.	Proteins can be separated by gel electrophoresis.
Hydrophilic	Water loving.	Sugar is hydrophilic and can dissolve in water.
Hydrophobic	Water hating.	Oil is hydrophobic and will not mix with water.
in vivo	Latin for "in life".	The experiment was performed *in vivo*.
Lead Compounds	The first chemical compounds to be the starting point for a drug.	A lead compound for a new painkiller was discovered in the bark of a willow tree.
Mitochondria	The powerhouse of the cell.	One of the cells organelles is the mitochondria.
Organelle	A cell's organs.	The nucleus is a cell organelle.
Pathogen	A virus, bacteria, parasite or fungus that causes illness.	They were infected with a pathogen and got sick.
Pharmaceutical	Any chemical made with a medicinal effect.	The pharmaceutical industry makes medicines.
Pharmacokinetics	Where a drug goes in your body and how long it stays there.	To understand how a drug works we must first look at pharmacokinetics.
Spectrometry	Measuring how light interacts with chemicals.	We can use spectrometry to measure how much light a chemical can release.
Subshell	A subdivision of an electron orbital.	The electron orbitals 2,8,8,8 can be divided into subshells; s, p and d.

Tutorial 1 – Poisons, Medicines and Chemicals

What is the Purpose of Tutorial 1?

This tutorial will get you thinking about the relationship between chemicals and us. What is the difference between poisons and medicines? Can substances normally classified as food, have a medicinal benefit?

These questions are important to consider and by the end of this tutorial you will have a clear idea of how we define these substances. You will also start to understand the first steps towards discovering and designing medicines and learn some historically famous drugs and their discovery!

By the end of this tutorial you should be able to:

- Define what a drug is i.e. chemicals vs poisons vs food
- Define what a disease is, and comment on how medicines can treat virus, bacteria and non-communicable diseases.
- Be able to explain where Lead Compounds are found.

Notes

Activity 1 – Poison or Food?

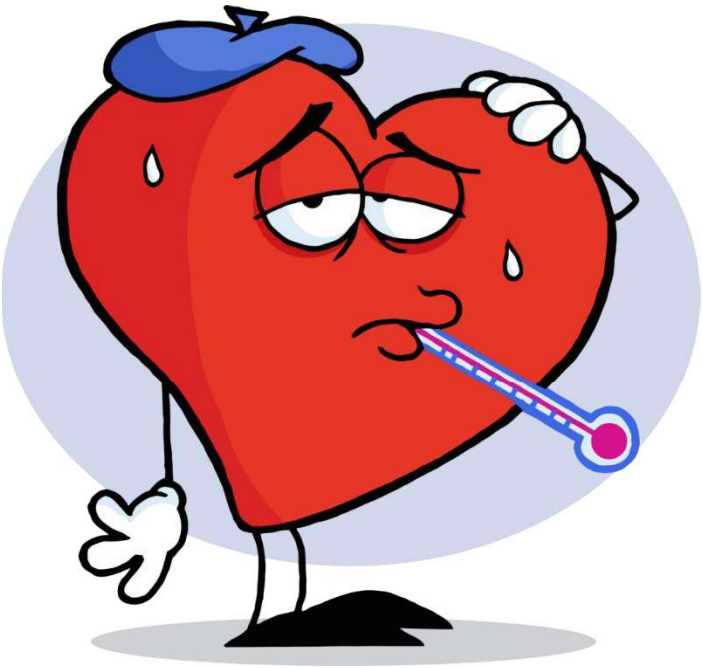
What is the definition of a chemical?

What is the definition of a poison?

What is the definition of a medicine?

What is the definition of food?

Use this space to glue in the cards used in the activity.



Activity 2 – When Living Goes Wrong

In pairs discuss the answers to these questions.

What is a disease?

Can we name any diseases?

Have you ever been sick and received medicine?

Can you remember the name of that medicine?

Is there a medicine for every disease and illness?

Is there money to be made from diseases?

In what ways can chemicals be discovered that become lead compounds for new medicines?

Activity 3 – How to Discover Information

Using the space below draw a mind map detailing the medicines that you know and their uses. This mind map will be the basis for the baseline assignment.

Tutorial glossary

Use this space to write down any words you have learned today

Word	Definition	In a sentence

Notes

Homework 1 – Baseline Assignment

Write 500 words on a drug /medicine of your choice explain its purpose, history, and target disease.

Use the mind map to help guide your essay.
In appendix 2 you will find a blank space, this is for sticking in a data set used to determine drug efficacy. Do this once you've determined what drug you're researching.

Areas that would be worth including might be:

- Explanation of what makes something a drug, and not a poison
- The history of your drug
- The action of your drug
- The efficacy and success of the drug

Any other interesting points or arguments will be given bonus marks.

Success Criteria

A high-quality assignment will correctly use key phrases found in the glossary and indicate that you can research and reference correctly.

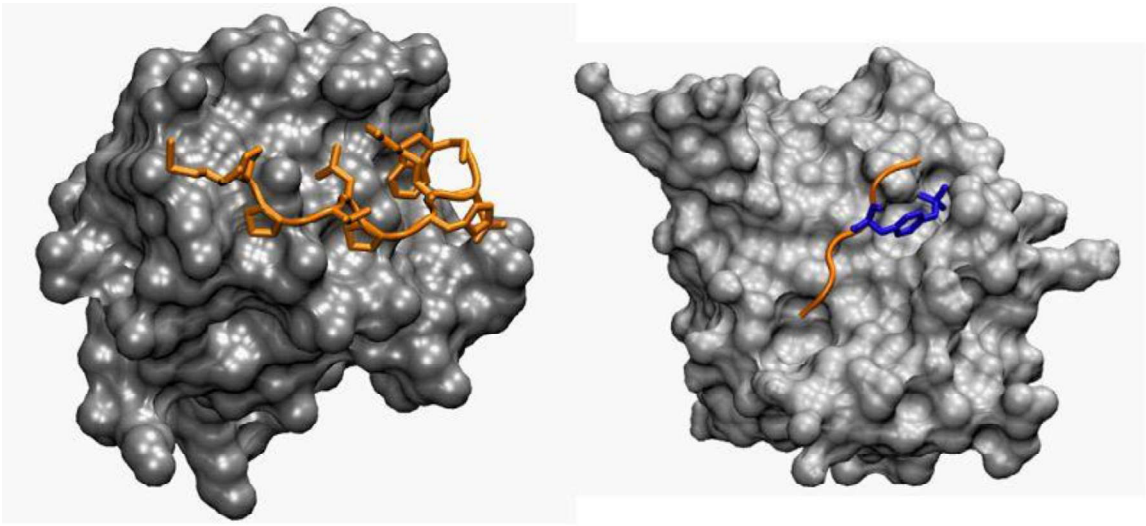
Try to include 2 – 3 references, see appendix 1 for guidance on how to do this.

Give reasons and evidence to back up the points that you make. You might find the PEE structure helpful:

- Point – make the key point clear
- Evidence – explain the evidence for the point
- Explanation – explain why your point is relevant and important

Organise your writing into paragraphs, include an introduction and conclusion.

Tutorial 2 – The Shape of Drugs



What is the Purpose of Tutorial 2?

In this tutorial, we will recap some of the principles of drug design we discussed in tutorial 1. We will go over the baseline assignment and discuss what went well, and look at how to improve on the next assignment.

We will look at the covalent bond and see how the interaction of a medicine with a protein is dependent on the shape of the chemical and not its reactivity.

By the end of this tutorial you should be able to:

- Recall a case study where a Lead Compound had been found
- Illustrate how covalent bonds build into drug shapes
- Define how drug shapes interact in the body e.g. Lock and Key Model

Activity 1 – Lead Compounds

Using the feedback from your baseline assignment, discuss in pairs the following questions.

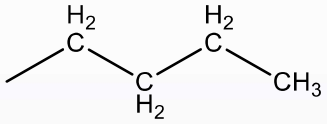
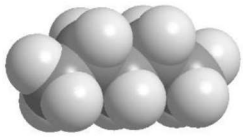
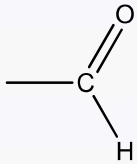
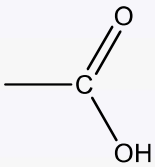
What sources are used in the discovery of lead compounds?

How do medicines interact with the body?

What methodology do we use to decide whether a medicine works or not?

Activity 2 – Covalent Bonds

Use this space to draw the functional groups and their equivalent organic shapes.

Displayed Functional Group	Name	Shape
	Alkane	
		
		
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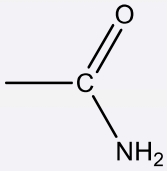
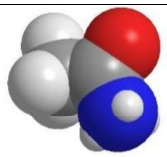
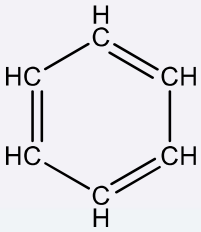
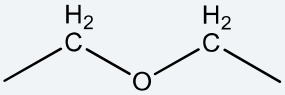
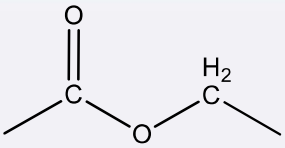
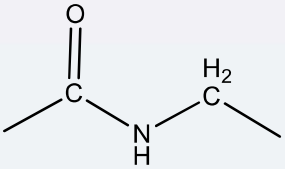
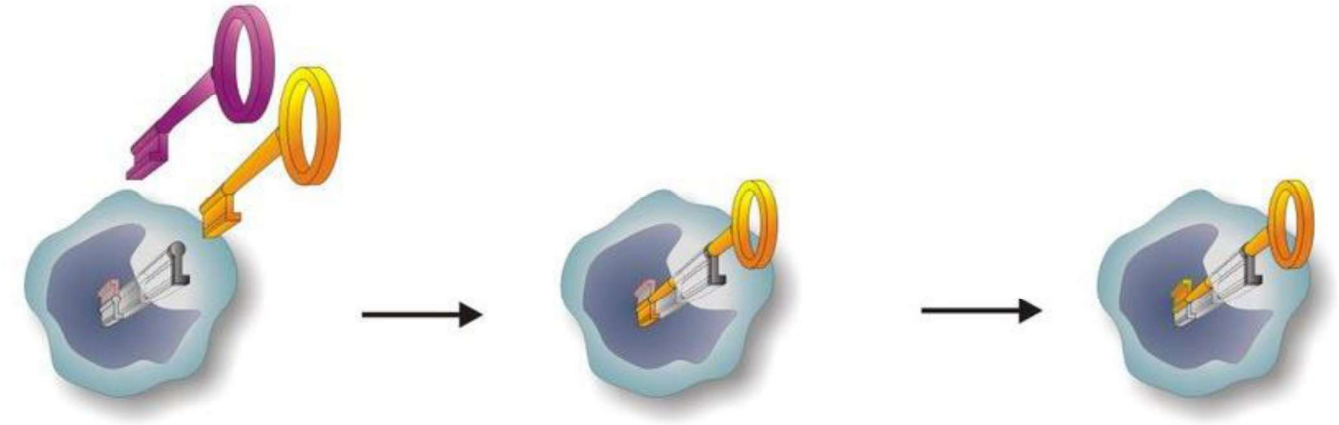
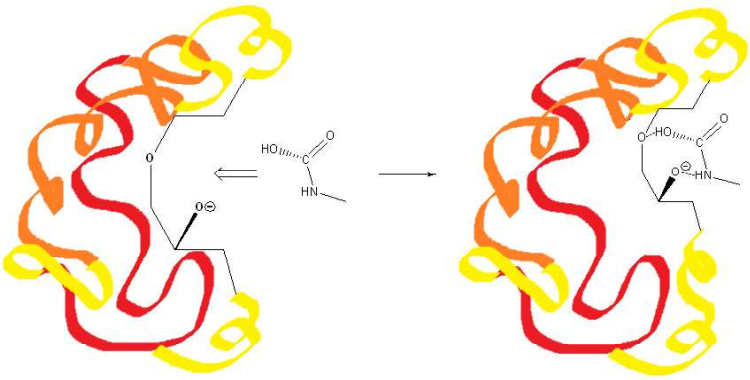
	Primary Amide	
	Benzene	
		
		
		

Activity 3 – Lock and Key Model



Using functional groups, draw the correct "key" to fit the protein pocket shapes. An example is done for you.



Tutorial glossary

Use this space to write down any words you have learned today

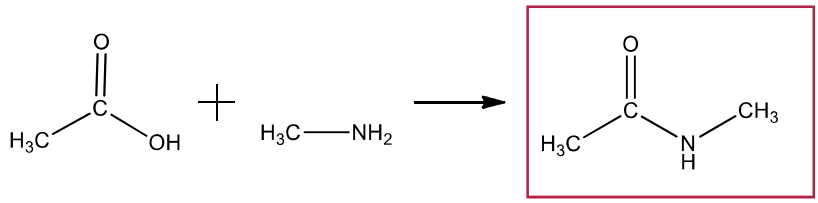
Word	Definition	In a sentence

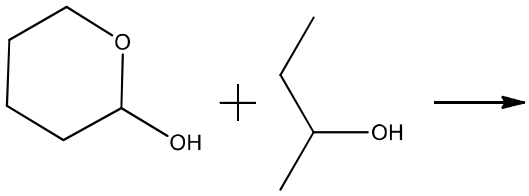
Notes

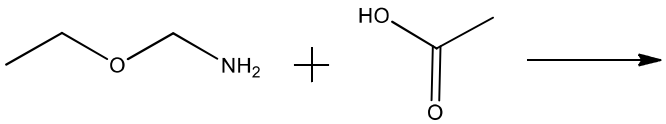
Homework 2 – Couple these Compounds

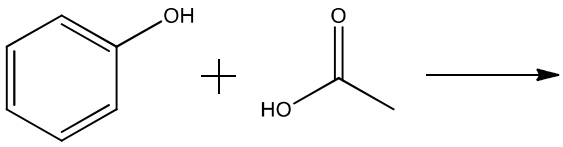
When two compounds couple together, often water is released. This is called a condensation reaction and is used in joining lots of different chemicals together including amino acids to form proteins, and sugars to form carbohydrates.

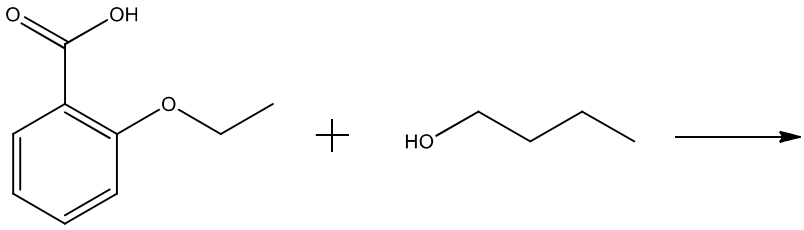
Draw these compounds coupled together with an ester or amide bond. Successful completion of this homework will see the compounds redrawn and coupled together releasing one H_2O molecule. The first one has been done for you.

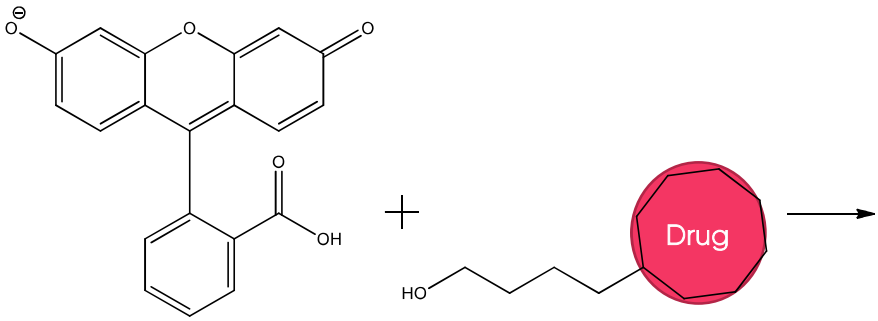




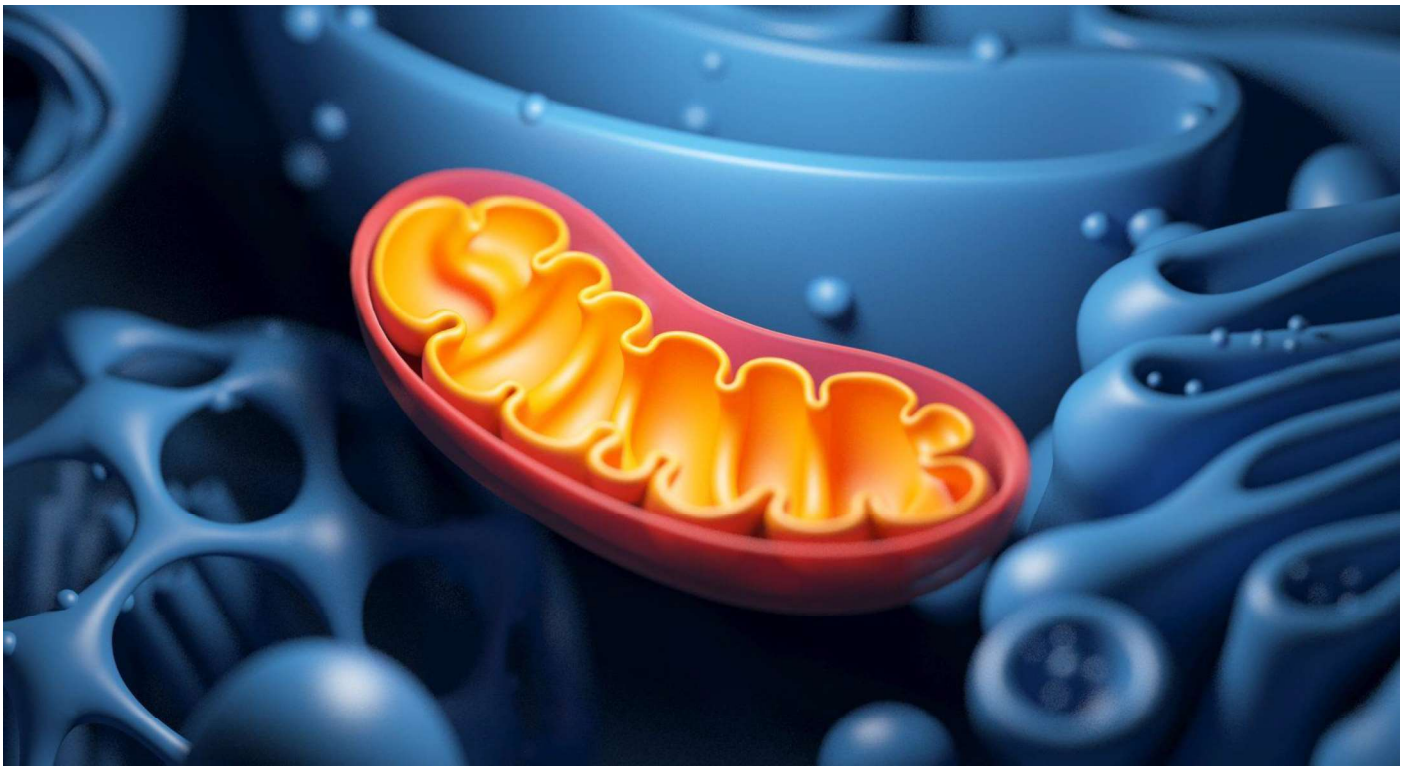








Tutorial 3 – The Creature Inside your Cells



What is the Purpose of Tutorial 3?

In this tutorial we will start and look at the biology of animal cell, mitochondria and the events that lead to damage in a heart attack.

By the end of this tutorial you should be able to:

- Draw an animal cell featuring some of its organelles namely:

  mitochondria, nucleus, bilayer cell wall, channel proteins.

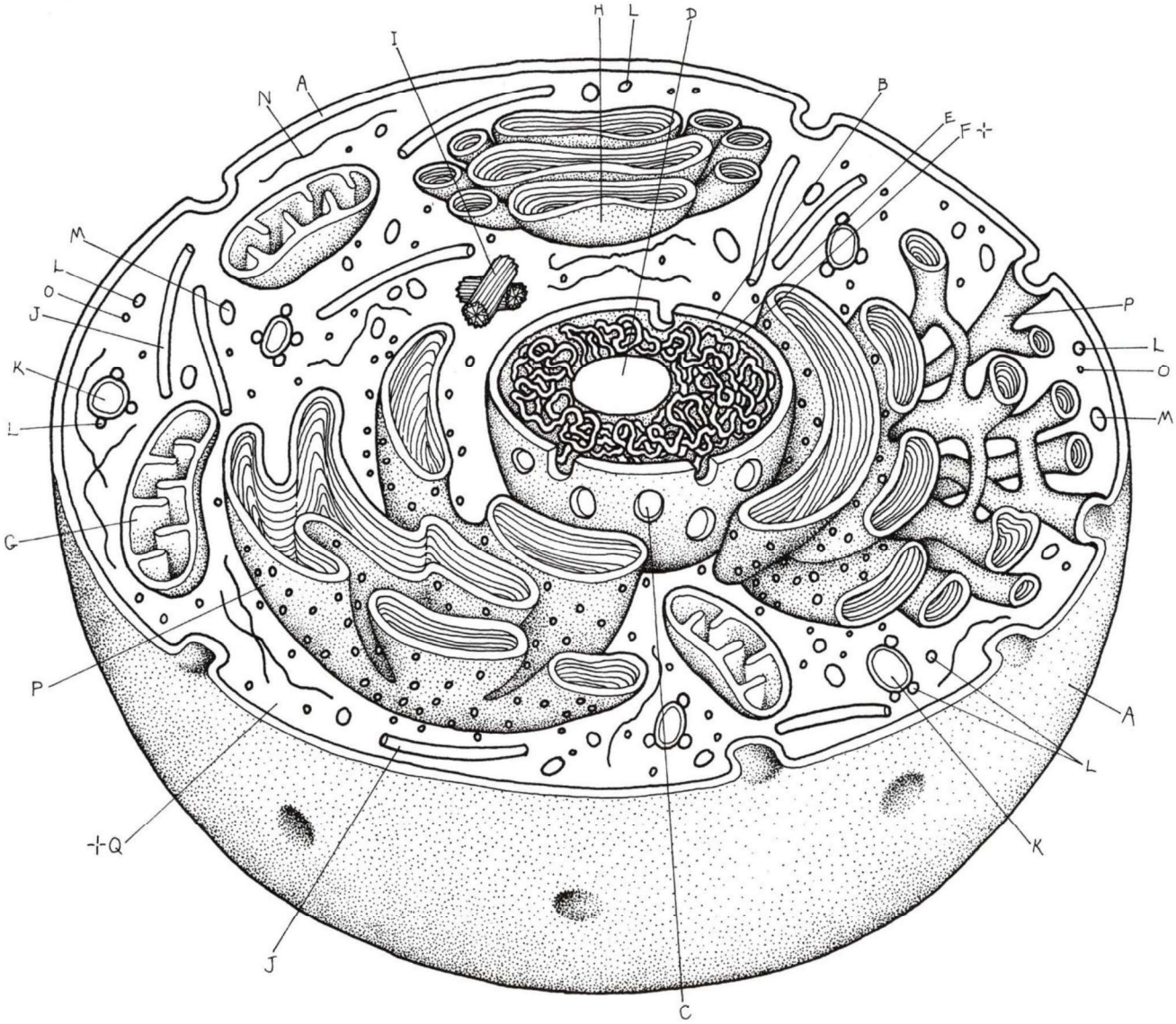
- Explain the role of the mitochondria in generating power for the cell.
- Answer University level questions on the behaviour of mitochondria during a heart attack - reperfusion injury.

Activity 1 – The Structure of an Animal Cell

ANIMAL CELL

CELL MEMBRANE
NUCLEUS
NUCLEAR ENVELOPE
NUCLEAR PORE
NUCLEOLUS
CHROMATIN
NUCLEAR SAP
CYTOPLASM
MITOCHONDRION
GOLGI COMPLEX

CENTRIOLE
MICROTUBULE
VACUOLE
LYSOSOME
MICROBODY
MICROFILAMENT
RIBOSOME
ENDOPLASMIC RETICULUM
HYALOPLASM



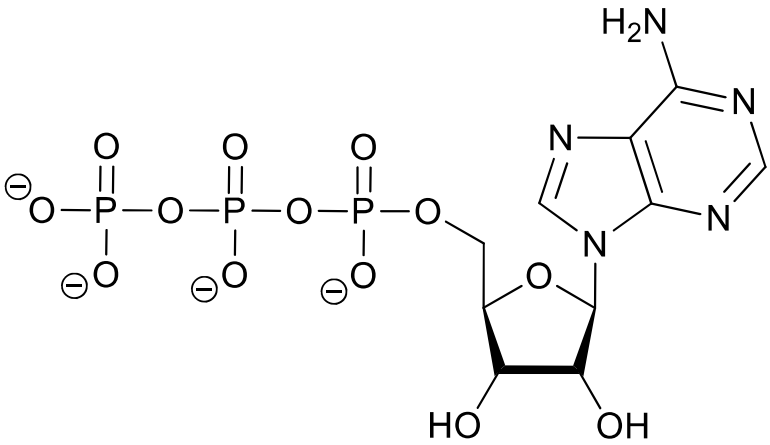
Activity 2 – The Electron Transport Chain

Mitochondria produce the energy for the cell. In pairs, discuss about what you think the body uses for energy.

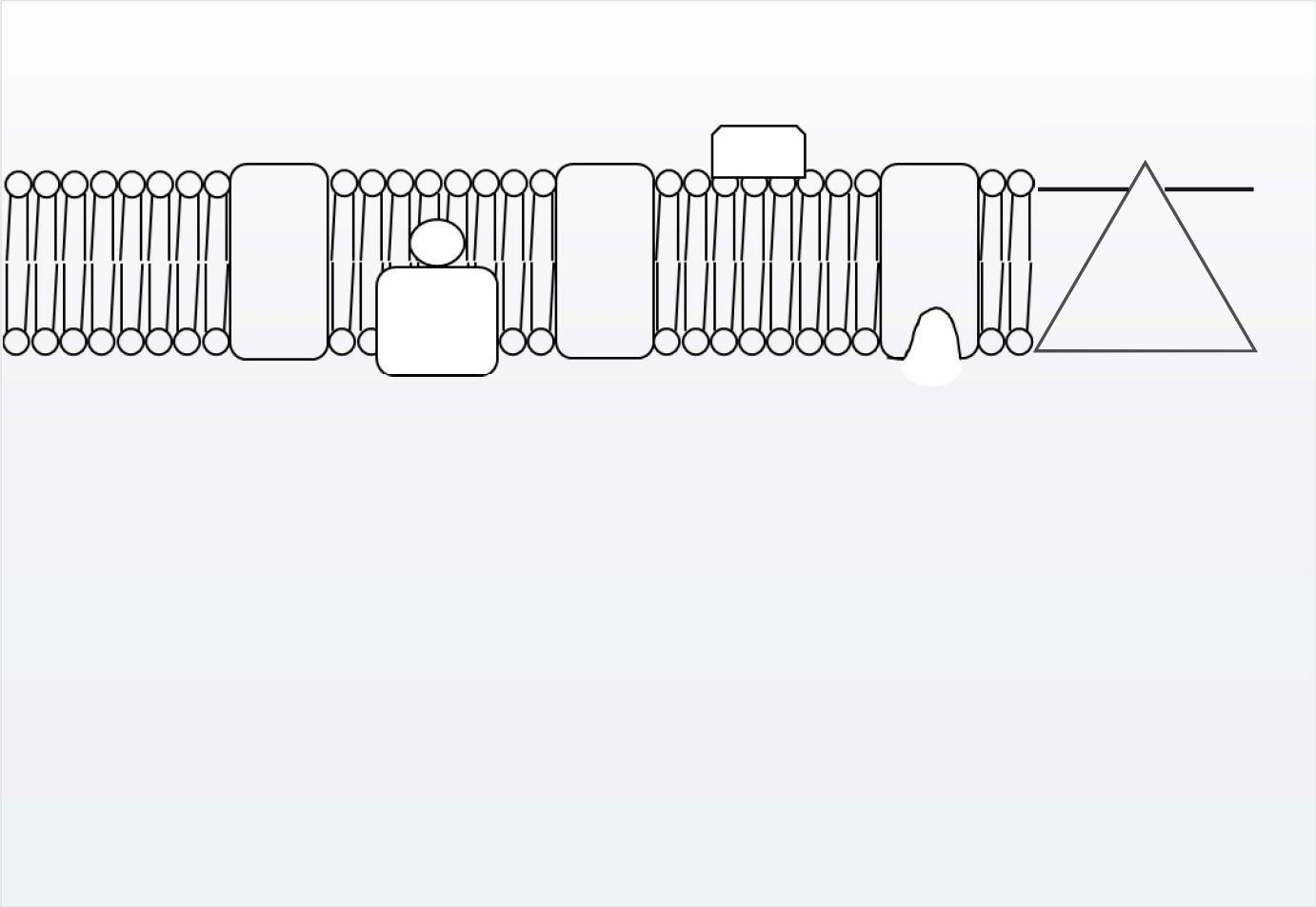
Complete the word equation for the production of ATP.

_____ (ADP) + _____ ⟶ Adenosine Triphosphate (_____) + _____

The structure of ATP can be found below, annotate the diagram labelling the components.



Below is a partially filled diagram of the electron transport chain. Fill in the rest of the diagram using the information given on the screen.



Activity 3 – What Happens Inside the Cell During a Heart Attack?

What are the risk factors that lead towards cardiovascular disease?

Detail step-by-step the stages of a heart attack.

What is the behaviour of mitochondria during a heart attack?

Define the term 'free radical'.

Draw a diagram of a heart attack and show how a stent is used to restore blood flow to the heart.

Tutorial glossary

Use this space to write down any words you have learned today

Word	Definition	In a sentence

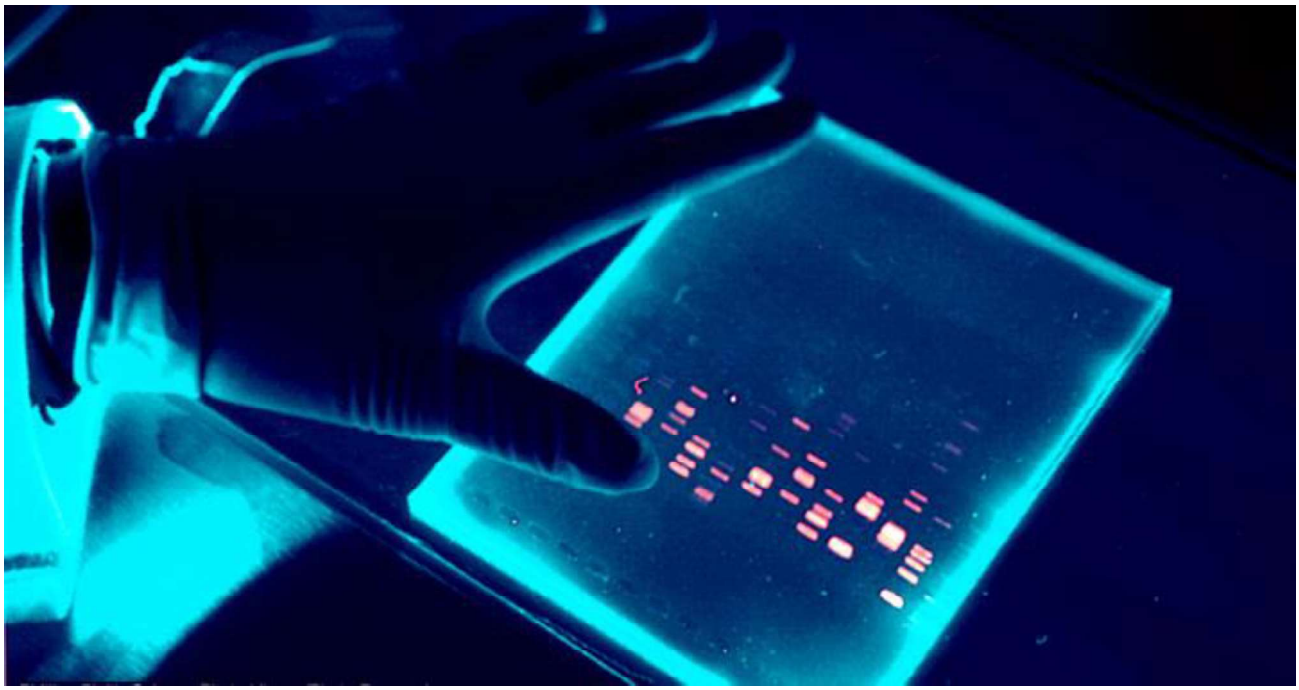
Notes

Homework 3 – Poster Illustrating the Stages of Reperfusion Injury

For this homework, draw the stages of a heart attack and how it leads to reperfusion injury. Put the information you discover into a poster with images.

A successful poster will feature sections on risk factors for heart disease, the role of the mitochondria in a heart attack, and how reperfusion injury causes damage to the heart.

Tutorial 4 – Glow in the Dark Drugs



What is the Purpose of Tutorial 4?

This tutorial is an opportunity to engage in University level practical work. We will attempt to couple a fluorescent marker (fluorophore) to a new lead compound called Risoxitam. This drug-fluorophore compound has been incubated with a tissue sample and we will analyse how much of the drug has bound to the cell targets.

We will then look at gel electrophoresis and mass spectrometry to analyse proteins that have interacted with Risoxitam.

By the end of this tutorial you should be able to:

- Describe how some commonly used biomedical techniques can be used in the synthesis of new drugs.
    - Gel electrophoresis
    - Mass spectrometry
    - Activity Assay
- Perform organic synthesis reactions.
- Explain how fluorescence can be used to identify cellular components.

Activity 1 – Coupling Fluorophore to a Drug

This activity is a practical experiment where you will attempt to couple a new lead compound to a fluorescent marker.

Write the chemical equation.

Next, detail the glassware to be used and draw the image of the set up.

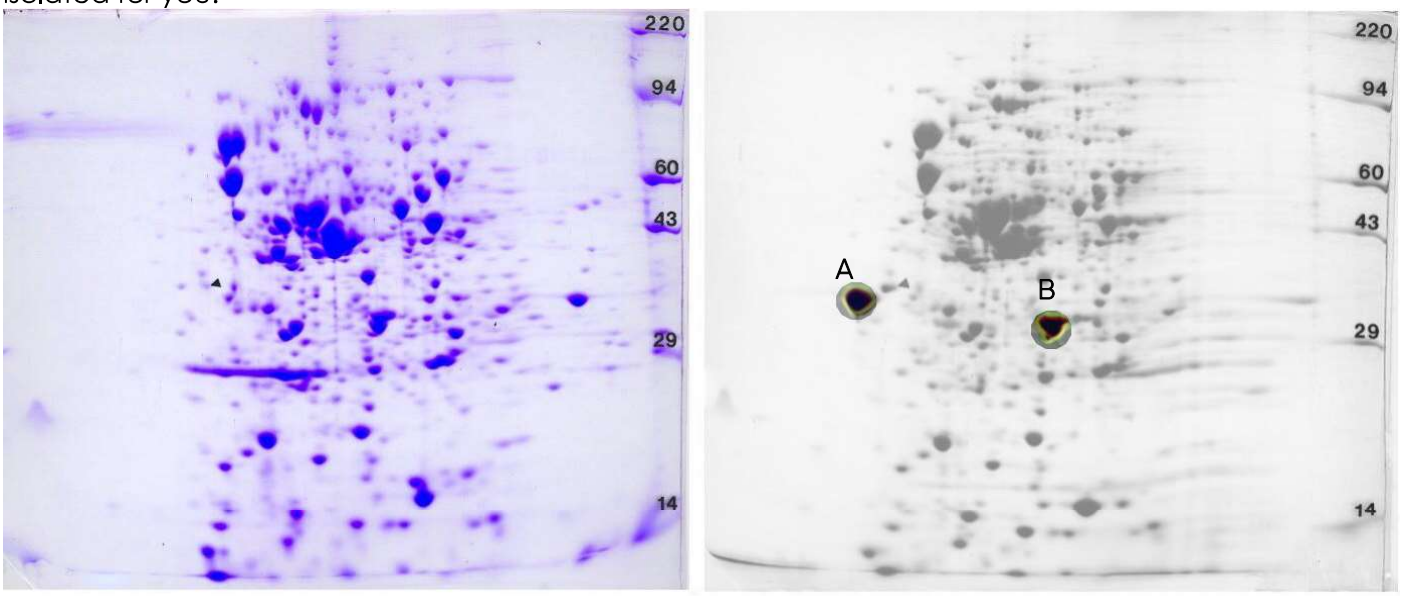
Equipment

What are the associated hazards and which precautions need to be taken?

Method

Activity 2 – Gel Electrophoresis Analysis

Below is an example of a 2D gel electrophoresis blot. Two spots are fluorescent and have been isolated for you.

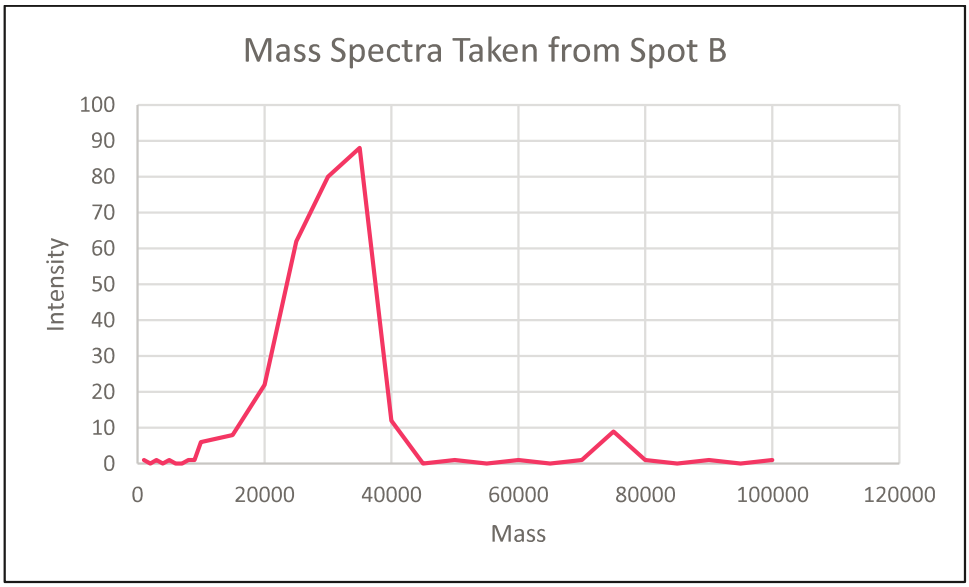


Draw the locations of the fluorescent spots on the left gel image.

Why do you think only one spot is easy to locate on the left gel?

Why does spot A disappear when all the proteins are stained?

Spot B is picked out, and the proteins are extracted. This extracted mixture is then injected into a mass spectrometer and the weight is revealed. Look at the spectrum below and identify the protein using the data in appendix 5.



Activity 3 – Activity Assay

An activity assay is used to determine how active a drug is within a target. This can be measured in almost anything: protein, enzyme, cell, tissue or living organisms. You are given a tissue sample that has been incubated with the fluorophore coupled to Risoxitam. You need to determine how much of the drug has bound to the tissue sample. This will be done with spectroscopic measurements to see how brightly the sample fluoresces.

Outline the method below.

Use this blank table to record your results.

Calibration Concentration mg/ml	Tissue Sample	Intensity A.U.
	1	
	2	
	3	

Tutorial glossary

Use this space to write down any words you have learned today

Word	Definition	In a sentence

Notes

Homework 4 – Crushing Numbers

Process the spectroscopic data received in this tutorial into a calibration graph. This should be done with Excel or another graph drawing program e.g. Google sheets.

Use the graph to find the equation of the line in the format of $y = mx + c$ and use it to determine the concentration of the drug bound to the tissue sample.

Note that the equation will have to be rearranged in order to do this!

Success Criteria

1. A clearly and correctly labelled graph.
2. The equation of the graph and the R^2 value clearly shown.
3. A trendline that demonstrates an increase in fluorescence proportional to the concentration of the fluorescent drug.
4. Graph processed with either Excel or another graph drawing process. (If you need help with this, I can show you in breaks or after school BEFORE the deadline).

Use the space below to write step-by-step instructions on rearranging $y = mx + c$ and how to use MS Excel.

Tutorial 5 – How We Know Our Medicines Work

What is the Purpose of Tutorial 5?

Throughout this tutorial series we have looked at medicines, how they are designed, and even attempted a practical experiment to determine the efficacy of a new drug that will hopefully make it to market as a heart attack preventative.

Before we can move on to the final assessment it is worth considering how our drugs fluoresced. We need to understand the physics of light emission from electrons. In activity 1 we will study the emission of light from fluorescent chemicals.

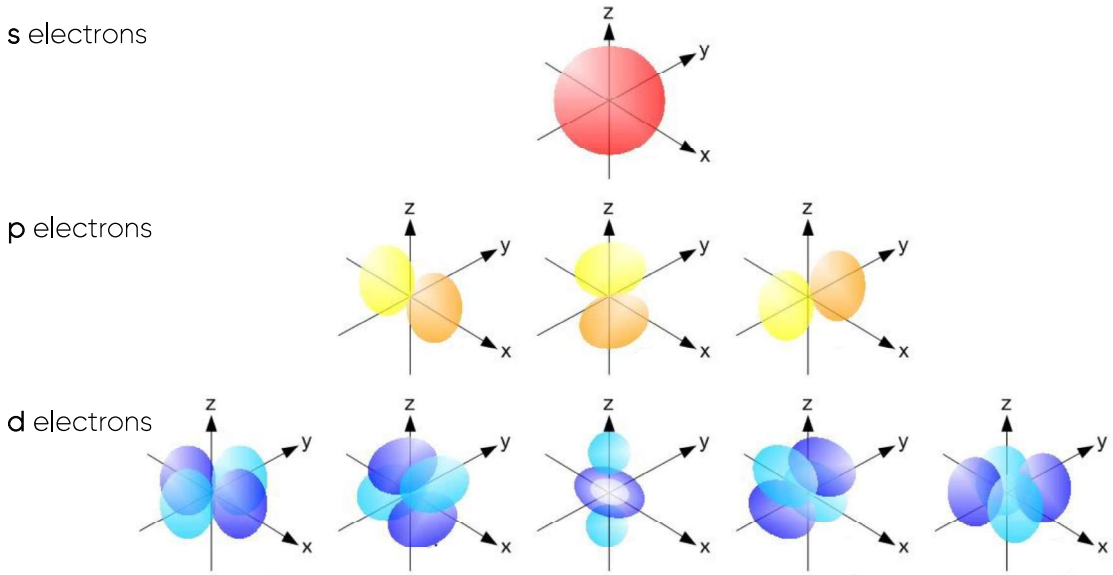
Once scientists know a drug might work, the lead compound can move onto preclinical trials. This is a key area of knowledge that we will study.

By the end of this tutorial you should be able to:

- Communicate about how chemicals can emit light as fluorescence
- Write a high-level answer for the process of drug design *in vivo* and preclinical trials.

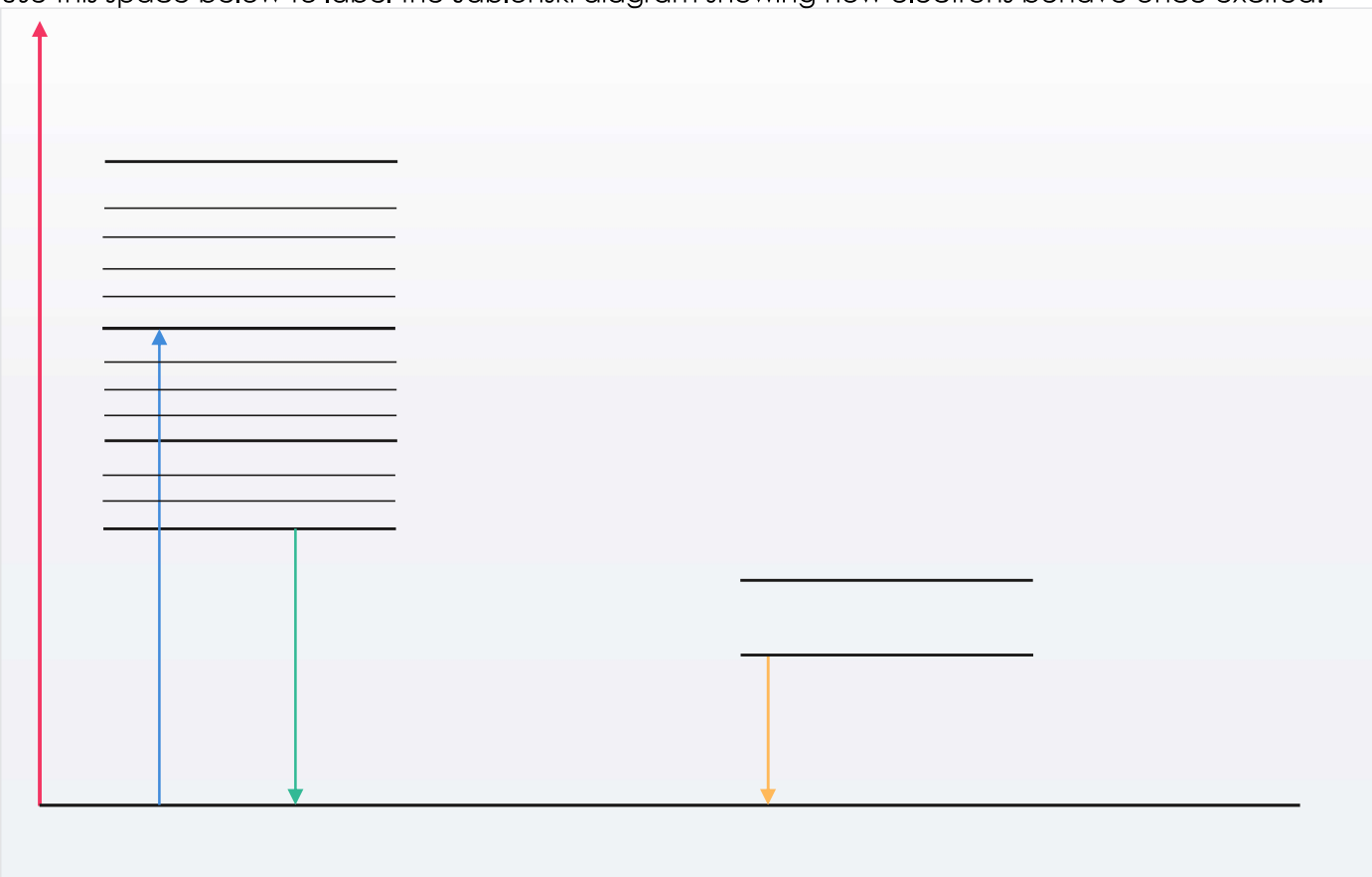
Activity 1 – The Jablonski Diagram

Electron Subshells



This image shows the areas around an atom that electrons like to move around in. How do electrons behave when they are excited with excess energy?

Use this space below to label the Jablonski diagram showing how electrons behave once excited.



Activity 2 – Step by Step Preclinical to Clinical Trials

The following questions are common GCSE exam questions about drug trials. We shall be looking at the answers to these questions throughout this activity.

1. What are the steps to getting a drug to market?

2. New drugs must be tested and trialled before being used. What are new drugs tested on in a laboratory?

3. Why is it important that a drug is trialled before being given to patients?

4. What is a double-blind trial and why is it used?

Activity 3 – Mind Map and Planning for Final Assessment

Tutorial glossary

Use this space to write down any words you have learned today

Word	Definition	In a sentence

Notes

Homework – Final Assignment

Write a 2000-word essay on the new cardioprotective drug we experimented on in tutorial 4. Use the lab report template in appendix 3 as your guide.

Include details of:

- The availability of the lead compound. Where does the lead come from? Can it be harvested, or will it be made synthetically in a lab?
- What you have discovered about the efficacy of the drug, how well does it bind to its target?
- Include a "further work" section detailing further biological assays and chemical tests you can perform to determine the drugs pharmacokinetics and efficacy.
- What are the clinical trials this drug would then go through assuming it is successful in preclinical trials?

An essay at a 1st class pass level will meet the following success criteria:

- use correct phrases and keywords from the glossary.
- plot and analyse data sets processed in tutorial 4.
- reference the techniques to outside resources.
- use the lab report format correctly.
- include an evaluation of suitable techniques, with clear justification and reasoning.
- give a conclusion that comments on the efficacy on the drug.
- use of SPAG correctly.

Appendix 3 includes details and a guide about what your essay will look like. For an example of technical writing see appendix 7.

Tutorial 6 – Feedback tutorial

What is the Purpose of Tutorial 6?

- To receive feedback on your final assignment
- To respond to the feedback from your Uni Pathways teacher
- To write targets for improvement on your final assignment

Final assignment feedback from your Uni Pathways Teacher

(Remember to look at the mark scheme to help you understand what you have done well so far, and how you can do even better in your final assignment)

Here are three things that my Uni Pathways Teacher thought I did well in my draft assignment
•   •   •

Here are three things that my Uni Pathways Teacher thinks that I could do to get a higher mark in my final assignment
•   •   •

Tasks from my Uni Pathways Teacher to do during the feedback tutorial to help me improve
•   •   •
My response:

Actions I will take to improve my final assignment after this tutorial…
•   •   •

Hand in date for my final assignment:	

Tutorial 7 – Final tutorial

What is the Purpose of Tutorial 7?

- To receive feedback and a grade on your final assignment.
- To reflect on the programme including what you enjoyed and what was challenging.
- To ask any questions you may have about university.

Final assignment feedback from my Uni Pathways Teacher

Final mark:	University style grade:
Feedback: Here are three things that my Uni Pathways teacher thought I did well in my final assignment • • •	

Here are three things that my Uni Pathways teacher thinks I should remember for when I am doing this kind of study in the future
• • •

University

What questions do you still have about University after taking part in Uni Pathways?
•   •   •

Reflecting on Uni Pathways

What did you most enjoy about Uni Pathways?
•   •   •

What did you find challenging about the programme?	How did you overcome these challenges?
• • •	• • •

Appendix 1 – Referencing correctly

When you get to university, you will need to include references in the assignments that you write. This is really important, because it will help you to avoid plagiarism. Plagiarism is when you take someone else's work or ideas and pass them off as your own. Whether plagiarism is deliberate or accidental, the consequences can be severe. In order to avoid losing marks in your final assignment, or even failing, you must be careful to reference your sources correctly.

What is a reference?

A reference is just a note in your assignment which says if you have referred to, or been influenced by, another source such as book, website or article. For example, if you use the internet to research a particular subject, and you want to include a specific piece of information from this website, you will need to reference it.

Why should I reference?

Referencing is important in your work for the following reasons:

- It gives credit to the authors of any sources you have referred to or been influenced by.
- It supports the arguments you make in your assignments.
- It demonstrates the variety of sources you have used.
- It helps to prevent you losing marks, or failing, due to plagiarism.

When should I use a reference?

You should use a reference when you:

- Quote directly from another source.
- Summarise or rephrase another piece of work.
- Include a specific statistic or fact from a source.

How do I reference?

There are a number of different ways of referencing, and these often vary depending on what subject you are studying. The most important thing is to be consistent. This means that you need to stick to the same system throughout your whole assignment. Here is a basic system of referencing that you can use, which consists of the following two parts:

1. **A marker in your assignment**: After you have used a reference in your assignment (you have read something and included it in your work as a quote, or re-written it your own words) you should mark this in your text with a number, e.g. [1]. The next time you use a reference you should use the next number, e.g. [2].
2. **Bibliography**: This is just a list of the references you have used in your assignment. In the bibliography, you list your references by the numbers you have used and include as much information as you have about the reference. The list below gives what should be included for different sources.

    a. **Websites –** Author (if possible), title of the web page, website address, [date you accessed it, in square brackets].

    E.g. Dan Snow, 'How did so many soldiers survive the trenches?', http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [11 July 2014].

    b. **Books –** Author, date published, title of book (in italics), pages where the information came from.

    E.g. S. Dubner and S. Levitt, (2006) *Freakonomics*, 7-9.

    c. **Articles –** Author, 'title of the article' (with quotation marks), *where the article comes from* (newspaper, journal etc.), date of the article.

    E.g. Maev Kennedy, 'The lights to go out across the UK to mark First World War's centenary', Guardian, 10 July 2014.

Appendix 2 – Data for Baseline Assignment

Appendix 3 – Example Layout of Lab Report

Example title Candidates name Date of Experiment

Example Title of the Experiment

1.0 Introduction
[Background on the information, where the compound has been discovered, applications (disease treated), etc…]

2.0 Aims and Objectives
[What you intend to determine in this experiment, hypothesis - what do you expect to happen?]

3.0 Experimental
[Main title to contain the next sub titles below.]

3.1 Hazards Risks
[Give a brief summary of the experiment's risks]

3.2 Method
[Example method]

3.3 Results
[Any data, numerical processing and images from your experiment]

3.4 Discussion
[Accuracy of the experiment, problems you had and ways of overcoming them]

4.0 Conclusion
[Evaluate whether you achieved what you intended to achieve as stated in your introduction and include a brief summary.]

4.1 Further Work
[What other experiments and assays could you do to prove the drugs' efficacy? Is this drug worth taking into clinical trials? What are those trials?]

5.0 References
[information to answer questions and method and where you found it]

Appendix 4 – Summary Poster

Appendix 5 – Protein Data

Entry Name	Protein Name	Organism	Mass
DWORF_HUMAN	Sarcoplasmic/endoplasmic reticulum calcium ATPase regulator	Homo sapiens (Human)	3,836
BAGE5_HUMAN	B melanoma antigen 5	Homo sapiens (Human)	4,711
MLN_HUMAN	Myoregulin	Homo sapiens (Human)	5,194
PRCD_HUMAN	Photoreceptor disk component PRCD	Homo sapiens (Human)	6,007
RPAB4_HUMAN	DNA-directed RNA polymerases I, II, and III subunit RPABC4	Homo sapiens (Human)	7,004
TM14A_HUMAN	Transmembrane protein 14A	Homo sapiens (Human)	10,712
ATIF1_HUMAN	ATPase inhibitor, mitochondrial	Homo sapiens (Human)	12,249
IGFL1_HUMAN	Insulin growth factor	Homo sapiens (Human)	12,363
BNIP3_MOUSE	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3	Mus musculus (Mouse)	20,978
CANB1_HUMAN	Calcineurin subunit B type 1	Homo sapiens (Human)	19,300
B2CL2_HUMAN	Bcl-2-like protein 2	Homo sapiens (Human)	20,746
CD7_HUMAN	T-cell antigen CD7	Homo sapiens (Human)	25,409
BPIA5_MOUSE	BPI fold-containing family A member 5	Mus musculus (Mouse)	29,175
CA074_HUMAN	UPF0739 protein C1orf74	Homo sapiens (Human)	29,561
CCDC3_MOUSE	Coiled-coil domain-containing protein 3	Mus musculus (Mouse)	31,093
CDX2_MOUSE	Homeobox protein CDX-2	Mus musculus (Mouse)	33,476
AUHM_HUMAN	Methylglutaconyl-CoA hydratase, mitochondrial	Homo sapiens (Human)	35,609
ANKUB_HUMAN	Protein ANKUB1	Homo sapiens (Human)	56,600
ARHGG_HUMAN	Rho guanine nucleotide exchange factor 16	Homo sapiens (Human)	80,105

Appendix 7 – Example of Technical Writing

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1,3,5-Trisubstituted benzenes as fluorescent photoaffinity probes for human carbonic anhydrase II capture†

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The synthesis of small molecule based 1,3,5-trisubstituted benzenes for photo-mediated capture of human carbonic anhydrase II with visualisation by fluorescence is described.

The 'capture' of proteins by small molecules *via* irreversible cross-linking mediated by photo-irradiation is of interest in the field of proteomics (for reviews see ref. 1). The technique has the potential for profiling protein-binding by small molecules, an objective of importance both for basic cell biology and in pharmaceutical science. Capture compounds, or photoaffinity probes, are typically endowed with three functions comprising (i) a selectivity function, such as an enzyme inhibitor, (ii) a photo-cross linking group (capture function) and (iii) a sorting group to enable separation of the captured protein from biological mixtures, such as biotin or an alkyne for subsequent modification. The captured protein(s) can be isolated using streptavidin beads and identified by mass spectrometry or Western blotting (for examples see ref. 2). We are interested in developing methods for protein capture and visualisation without necessitating an isolation or chemical conjugation step (Fig. 1).[3]

Various 'tripodal' molecules have been employed for protein capture by small molecules, for example amino acids, peptides and trisubstituted aromatics including 1,2,4-trisubstituted benzene derivatives.[4] However, there are few examples of the use of 1,3,5-trisubstituted benzenes for this purpose.[5] Here we report that 5-amino dimethylisophthalate can be readily modified to produce compounds (**1**, **2**) suitable for fluorescence based monitoring of protein capture (Fig. 2) as exemplified by work on human carbonic anhydrases (HCA), increases in the level of some isoforms of which are indicative of diseases including those related to hypoxia (CA IX).

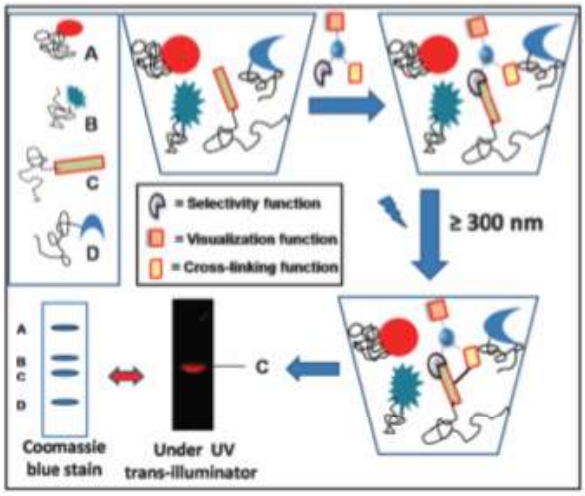

Fig. 1 Working principle of fluorescence based visualisation of capture.

We employed two sulphonamide derivatives that are known inhibitors for human carbonic anhydrase II (HCA II) as the selectivity function.[6] An aryl azide was chosen for photo-mediated cross-linking due to its ability to react irreversibly *via* nitrene formation.[7,8] A propargylated pyrene derivative was selected as a fluorescent visualisation function.

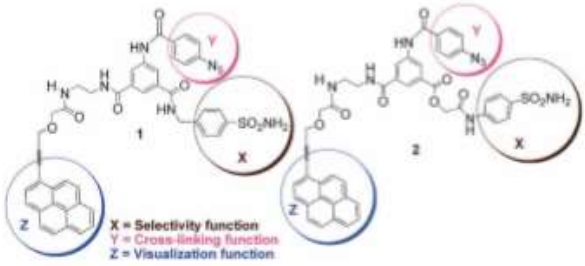

Fig. 2 Design of capture compounds.

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† Electronic supplementary information (ESI) available: Experimental procedure, compound characterization, copies of NMR, inhibition kinetics, ESI and MALDI-TOF MS. See DOI: 10.1039/c3cc38251f

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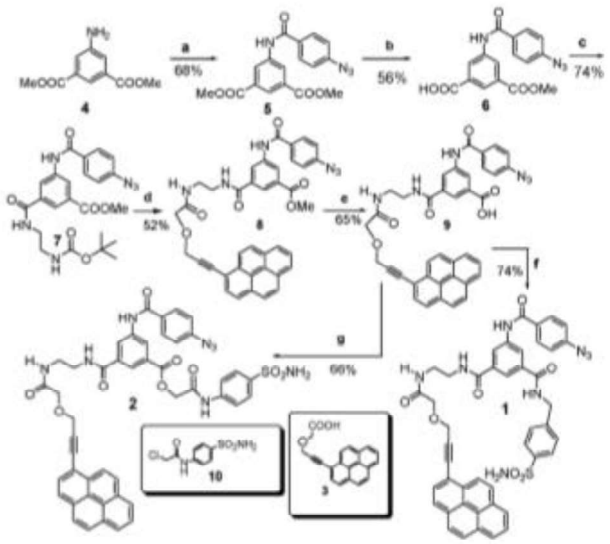

Scheme 1 Synthesis of capture compounds **1** and **2**. *Reagents and conditions*: (a) *p*-azidobenzoic acid, BOP-reagent, diisopropylethylamine (DIPEA), CH_2Cl_2 reflux, 24 h; (b) NaOH, MeOH, 50 °C, 3 h; (c) Mono-BOC protected ethylenediamine, BOP-reagent, DIPEA, CH_2Cl_2, 40 °C, 48 h; (d) (i) CF_3CO_2H, CH_2Cl_2, 0 °C – rt, 20 min; (ii) acid **3**, BOP-reagent, DIPEA, CH_2Cl_2 : DMF (5 : 1), rt, 20 h; (e) NaOH, MeOH, 50 °C, 6 h; (f) 4-aminomethyl-benzenesulfonamide, BOP-reagent, DIPEA, CH_2Cl_2 : DMF (5 : 1), 40 °C, 48 h; (g) K_2CO_3, 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **10**, dry DMF, rt, 20 h.

It was envisioned that the three groups could be attached to 5-amino dimethyl isophthalate acting as a tripodal template. A polar linker (diamino ethylene) was introduced between the visualisation group and the template in order to distance the former from the other two groups and to increase hydrophilicity (Fig. 2).

The synthesis started with BOP-mediated amide coupling with 4-azidobenzoic acid to link the cross-linking function to amino dimethyl isophthalate **4**. The product, **5**, was hydrolysed (NaOH) to give acid **6**. Subsequent coupling of **6** with mono-Boc protected ethylenediamine afforded amide **7**. Deprotection (CF_3CO_2H) followed by coupling of the resultant amine with acid **3** gave amide **8**, the ester of which was hydrolysed (NaOH). The resultant free acid **9** was coupled with 4-aminomethyl-benzenesulfonamide to furnish the capture compound, **1**. Reaction of **8** with chloro sulphonamide **10** using potassium carbonate in DMF produced the other capture compound **2** (Scheme 1).[9] The fluorescent tag **3** was prepared from 1-bromo-pyrene *via* a 3-step protocol (see ESI†).

We then investigated the inhibition of HCA II by **1** and **2**. Both were found to be reversible (competitive) inhibitors with compound **2** showing stronger inhibition (IC_{50}: **1** = 16.7 μM, **2** = 1.2 μM, for kinetic plots see ESI†). The issue of suitability of the template for the proposed capture compounds and the efficacy of the fluorescence based technique for visualising protein capture was then addressed. Thus, **1** and **2** were incubated with HCA II at various concentrations (15 min), irradiated (UV $\lambda \geq 300$ nm, 7 min) and then directly subjected to polyacrylamide gel electrophoresis (PAGE).[10] Exposure of the gel to UV-transillumination showed clear fluorescence bands at the expected region, which was further confirmed by Coomassie blue staining (Fig. 3).

The results validate compounds **1** and **2** as fluorescent capture compounds for HCA II. Capture is visible by in gel analysis under a UV-transilluminator down to HCA II concentrations of 4 μM, with the concentration of the capture compounds at 50 μM.

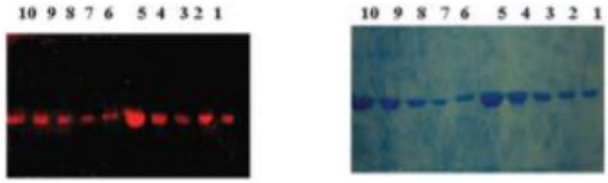

Fig. 3 Result of gel electrophoresis analysis of capture of HCA II by **1** and **2** at different protein concentrations, as visualised by UV (left) and Coomassie blue (right). Lanes 1–5 represent incubation with **1** (100 μM), lanes 6–10 represent incubation with **2** (100 μM). The final concentration of protein in lanes 1–5 and 6–10 was 4, 6, 8, 20 and 40 μM, respectively. HEPES buffer (pH 7.2) was used.

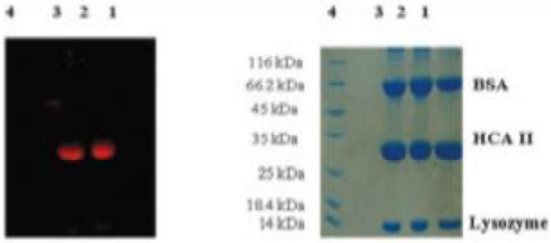

Fig. 4 Results of gel electrophoresis analysis of capture of HCA II in a mixture by **1** and **2** visualised using UV (left) and Coomassie blue (right). Lane 1: mixture of BSA, HCA II and lysozyme (all at 21 μM), lane 2: protein mixture (21 μM) + **1** (50 μM) with irradiation, lane 3: protein mixture (21 μM) + **2** (50 μM) with irradiation, lane 4: molecular weight marker. HEPES buffer (pH 7.2) was used.

We then investigated the selectivity of the probes. The process of incubation and photolysis was carried with a mixture of proteins *i.e.* HCA II, bovine serum albumin (BSA) and lysozyme. Under UV-transilluminator, only the fluorescence band corresponding to HCA II was visible, whereas Coomassie blue staining of the same gel showed bands corresponding to the three proteins (Fig. 4).

The experiment was repeated with cell lysates of *E. coli* where the selective capturing of HCA II was clearly apparent by PAGE (Fig. 5). Thus, both **1** and **2** showed high selectivity towards HCA II demonstrating the suitability of our 1,3,5-trisubstituted benzene template for capture and subsequent fluorescence-based visualisation.

The capture of HCA II by both compounds **1** and **2** was validated by MALDI mass spectrometric analyses. Thus, the incubated photo-reacted mixture was directly analysed using a MALDI-TOF mass spectrometer which revealed a new peak at *m/z* 29919.24 (**1**) or 29964.05 (**2**) corresponding to $[(M^+ (\text{HCA II}) + (\mathbf{1} \text{ or } \mathbf{2}) - N_2 + H^+)]$

Fig. 5 Gel electrophoresis of capture of HCA II from a cell lysate by **1** and **2** visualised using UV (left) and Coomassie blue (right). Lanes 1: 0.5 mg cell + **1** (10 μL, 1 mM) + 90 μL buffer (HEPES); **2**: 0.5 mg cell + **2** (10 μL, 1mM) + 90 μL buffer; **3**: 0.5 mg cell + 100 μL buffer.

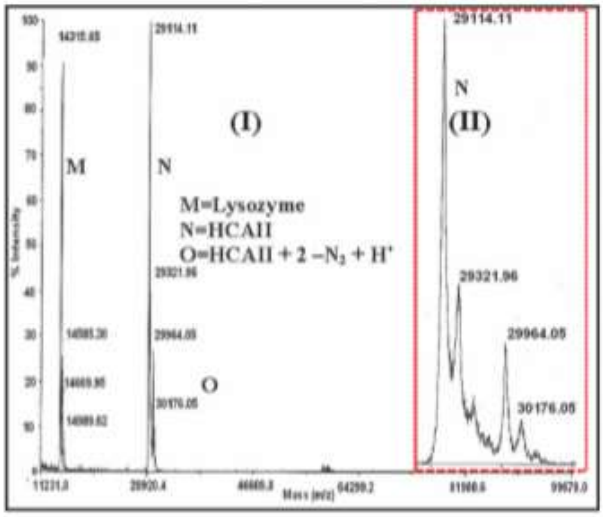

Fig. 6 MALDI spectra: (I) mixture of HCA II, BSA and lysozyme + **2**, incubated and photo-reacted; (II) expanded spectrum.

(see ESI†). The mass spectrum of the incubated photo-reacted mixture containing three proteins showed the peak corresponding to capture of HCA II only (Fig. 6 for capture by **2**; see ESI† for **1**).[11]

In conclusion, we have described two readily accessible small molecule probes, **1** and **2**, for selective capture of HCA II *via* photo-irradiation allowing fluorescent visualisation. The ability of 5-amino dimethyl isophthalate to act as a tripodal template for capture molecule design was demonstrated. We believe that 1,3,5-trisubstituted benzene based photo-reactive probes coupled with fluorescence based visualisation offer a simple and effective method for protein capture, which should be of utility in evaluating drug toxicity by studying their off-target interactions, inhibitor design and early disease diagnosis.

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9 Spectroscopic data: For **1**: δ_H (d_6-DMSO): 10.52 (1H, s), 9.21 (1H, t, J = 5.5 Hz), 8.66 (1H, bs), 8.42 (1H, d, J = 9.0 Hz), 8.39–8.27 (4H, m), 8.26–8.07 (5H, m), 8.02 (2H, d, J = 8.5 Hz), 7.77 (2H, d, J = 8.5 Hz), 7.49 (2H, d, J = 8.5 Hz), 7.22 (2H, d, J = 8.5 Hz), 4.72 (2H, s), 4.53 (2H, d, J = 5.5 Hz), 4.12 (3H, s), 3.16 (2H, s), 3.14 (3H, s). δ_C ($CDCl_3$): 169.6, 166.8, 166.7, 166.2, 144.2, 143.6, 143.2, 139.9, 135.9, 135.6, 131.9, 131.6, 131.3, 131.2, 131.0, 131.4, 129.5, 129.0, 128.3, 127.8, 127.4, 126.7, 126.6, 126.3, 125.5, 125.2, 124.2, 123.9, 122.9, 122.7, 121.7, 119.7, 116.8, 91.9, 85.5, 69.4, 59.5, 43.1, 38.5, 31.9; ν_{max} (KBr, cm^{-1}): 3752, 3413, 2923, 2857, 2364, 2122, 1651, 1599, 1547, 1452, 1333, 1284, 1155, 1099, 1028; λ_{max} (DMSO): 365 nm (ε 30 985 M^{-1} cm^{-1}), 347 nm (ε 24 630 M^{-1} cm^{-1}), 285 nm (ε 35 384 M^{-1} cm^{-1}); HRMS: calcd for $C_{45}H_{36}N_8O_7S$ + Na^+ 855.2325, found 855.2346. For **2**: δ_H (d_6-DMSO): 10.60 (2H, s), 8.83 (1H, s), 8.65 (1H, s), 8.57 (1H, s), 8.45 (1H, d, J = 8.5 Hz), 8.34 (1H, d, J = 7.0 Hz), 8.29 (2H, d, J = 9.5 Hz), 8.23–8.04 (8H, m), 7.76 (4H, bs), 7.25 (4H, bs), 4.99 (2H, s), 4.72 (2H, s), 4.14 (2H, s), 3.32 (4H, s); δ_C ($CDCl_3$): 169.4, 166.2, 165.4, 146.5, 143.9, 141.7, 139.1, 136.4, 136.1, 135.6, 134.9, 133.9, 133.6, 132.3, 131.9, 131.6, 131.4, 130.8, 130.1, 129.7, 129.2, 128.8, 128.5, 127.6, 127.2, 126.4, 126.3, 125.2, 125.0, 124.2, 123.7, 119.4, 119.3, 116.5, 91.7, 85.3, 69.2, 59.3, 56.2, 38.3, 34.7; ν_{max} (KBr, cm^{-1}): 3855, 3751, 3398, 2924, 2853, 2357, 2127, 1745, 1696, 1606, 1542, 1399, 1308, 1243, 1155, 1102, 1021; λ_{max} (DMSO): 365 nm (ε 27 369 M^{-1} cm^{-1}), 347 nm (ε 21 200 M^{-1} cm^{-1}), 285 nm (ε 34 615 M^{-1} cm^{-1}); HRMS: calcd for $C_{46}H_{36}N_8O_9S$ + Na^+ 899.2224, found 899.2259.

10 Capture experiment protocol: for capture experiments, the HCA II concentration was kept at 0.5 μM and the total volume was made up to 100 μL with buffer (20 mM HEPES; pH 7.2). HCA II and capture compounds (10–100 μM) were mixed by vortexing followed by centrifugation. For cell lysate preparation, 5 mL of induced, lag phase BL21(DE3)pLysS cells carrying plasmid pACA/HCA II were resuspended in 0.5 mL of buffer (50 mM Tris; pH 8.0, 50 mM NaCl, 10 mM EDTA, 1 mM dithiothreitol, 1 mM phenyl methanesulfonyl fluoride, 0.2 mM $ZnSO_4$), sonicated and centrifuged at 10 000 rpm for 10 minutes. The cell lysate thus obtained was mixed with the 50 μM capture compounds. To probe the selectivity of the capture compounds towards HCA II, BSA and lysozyme were used as controls. BSA and lysozyme were mixed at equimolar concentrations with HCA II. The exactly same method was followed as used with HCA II. The compounds were incubated with proteins for 15 min, then photo-irradiated with a medium pressure mercury lamp at ≥300 nm for 7 minutes followed by SDS-PAGE and mass spectrometric analysis.

11 BSA was not observed likely due to its high mass.

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