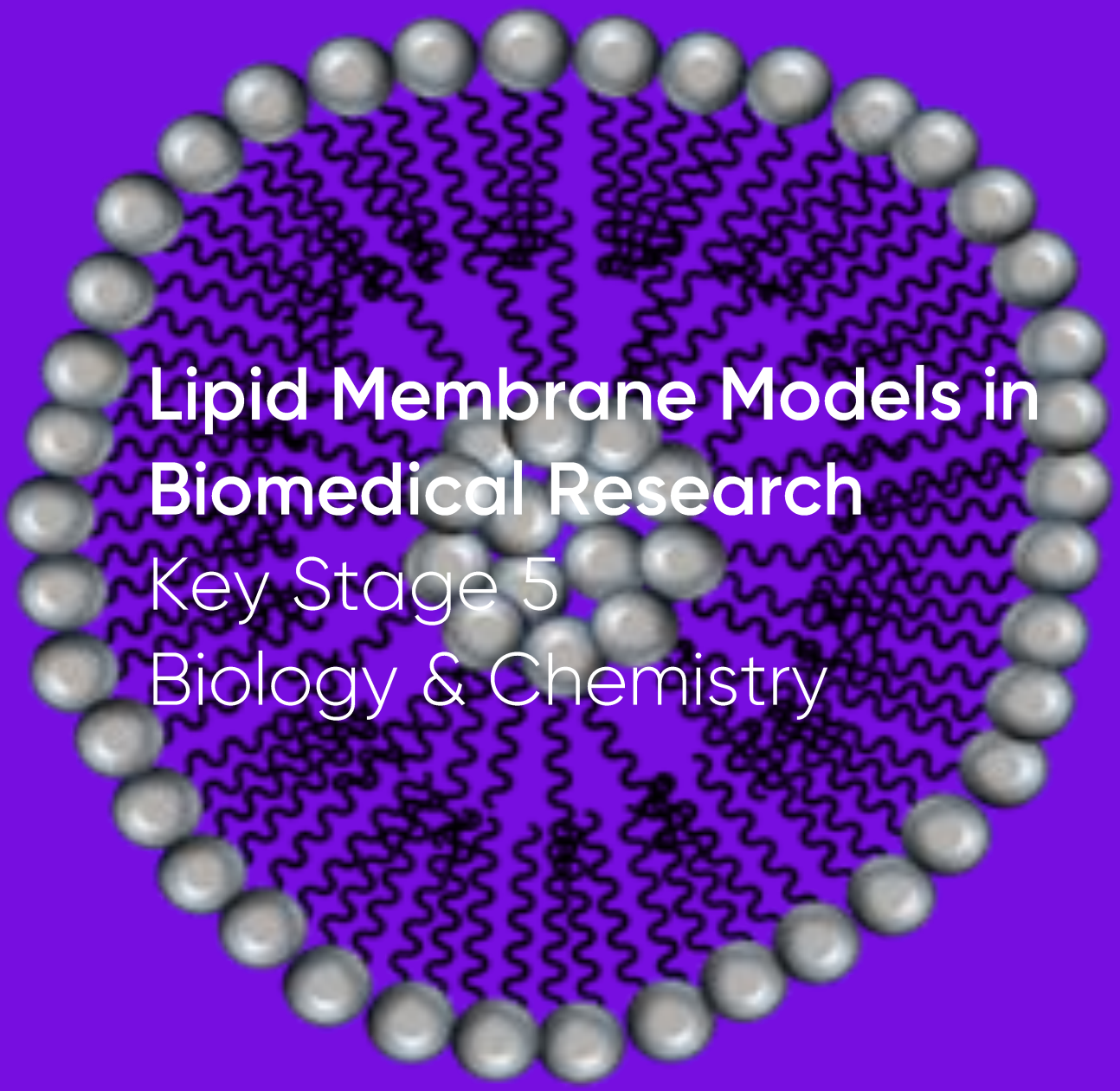
Research
Based
Curricula



Lipid Membrane Models in Biomedical Research

Key Stage 5
Biology & Chemistry

2020

BROUGHT TO YOU BY RBC ACCESS ED

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For Students
Getting Started

RBC means Research-Based Curriculum,. Each RBC coursebook is written by a PhD student at a university about their cutting edge research.

Why complete an independent 'RBC' study pack?

RBC courses are challenge courses to sharpen your skills and resilience: finishing a RBC course is a major accomplishment to add to your academic CV. To get into the university, you must demonstrate that you are intellectually curious, and will make the most of the academic opportunities available to you. Completing a pack will allow you to gain invaluable experience to write about in your university application..

It allows you to:

- ✓ Build your subject experience to mention in your UCAS Personal Statement
- ✓ Sharpen your academic skills
- ✓ Experience what it's like to study beyond school and at university
- ✓ Better understand what you enjoy and don't
- ✓ Improve your overall subject understanding ahead of final exams

For Students
Getting Started

What's in this booklet?

Your RBC booklet is a pack of resources containing:

- ✓ More about how and why study this subject
- ✓ Six 'resources' each as a lesson with activities
- ✓ A final assignment to gauge learning
- ✓ Extra guidance throughout about the university skills you are building
- ✓ End notes on extra resources and where to find more information

Who should complete this pack?

Anyone interested in improving their academic skills or understanding what they should do at university. *This pack is especially great for anyone interested in studying* ***Sciences,*** *particularly* **Biology and Chemistry,** *and want to understand how they link.*

Even if you are unsure of where your interest in these subjects can take you, by completing this pack you will have a clearer idea of the variety of subjects that link to one another.

If you have any questions while you are using the resources in this pack, you can contact your teacher or email us directly at schools@access-ed.ngo.

Good luck with your journey to higher education!

For Students
University Skills

To complete this resource, you will have to demonstrate impressive academic skills. When universities are looking for new students, they will want young people who can study independently and go above and beyond the curriculum. All of these skills that you will see here will demonstrate your abilities as a university student – while you're still at school!

Every time you have to look something up, or write up a reference you are showing that you can work independently.

Every time that you complete a challenging problem or write an answer to a difficult question, you might demonstrate your ability to think logically or build an argument.

Every time that you evaluate the sources or data that you are presented with, you are showing that you can "dive deep" into an unfamiliar topic and learn from it!

Skills you will build for university:

independent research	your ability to work on your own and find answers online or in other books
creativity	your ability to create something original and express your ideas
problem solving	your ability to apply what you know to new problems
building an argument	your ability to logically express yourself
providing evidence	your ability to refer to sources that back up your opinions/ ideas
academic referencing	your ability to refer to what others have said in your answer, and credit them for their ideas
Deep dive	your ability to go above and beyond the school curriculum to new areas of knowledge
source analysis	your ability to evaluate sources (e.g. for bias, origin, purpose)
Data interpretation	your ability to discuss the implications of what the numbers show
Active reading	your ability to engage with what you are reading by highlighting and annotating

Where can this subject take me?

Pathways

Studying Biology or Psychology can open the doors to many degrees and careers. It intersects with microbiology, chemistry, physiology, and sociology. Whatever interests you is likely to relate to biology in some way. See a snapshot of where studying Biology and Psychology can take you.

'Transferrable skills' from Biology to a career:

- research and data analysis
- problem-solving and creative thinking
- The ability to deliver successful projects
- communication, through report writing and presentations
- teamworking and collaboration
- the ability to work independently
- numeracy and maths
- IT and computer literacy

'Transferrable skills' from Chemistry to a career:

- strong mathematical/numerical ability,
- analysis and problem solving
- time management and organisation
- written and oral communication
- monitoring/maintaining records and data
- teamwork
- research and presentation

What are some are the 'interdisciplinary' subjects in this course?

Interdisciplinary is a term you will hear used by higher education institutions. It's also how many professionals and academics in the real-world operate: they use multiple subjects, or disciplines, to achieve their work.

By thinking about which subjects you like, alongside maths, it can help you choose a career pathway later.

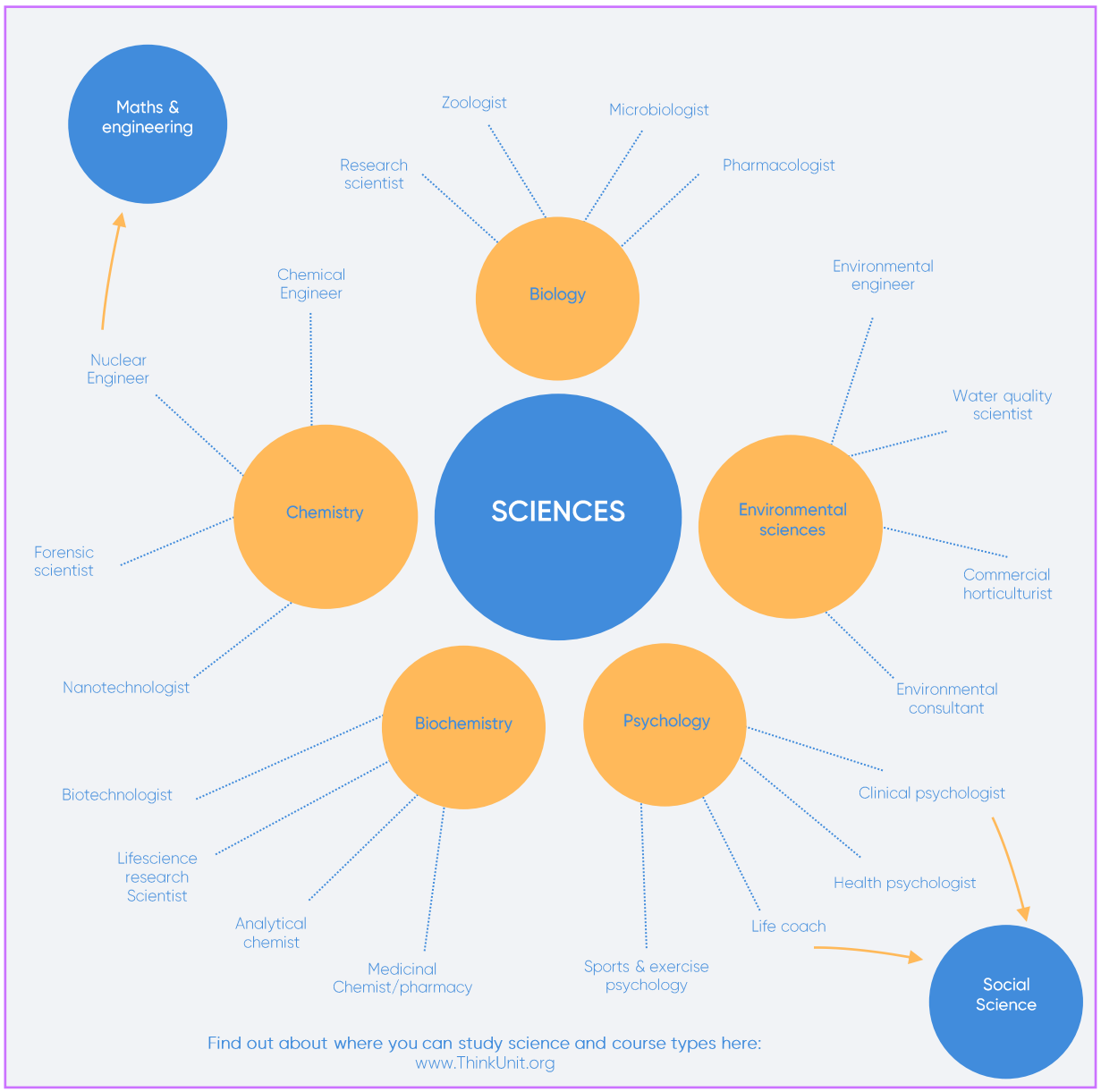
Read more about subject selection and careers pathways:

https://targetjobs.co.uk
https://www.prospects.ac.uk
https://thinkuni.org/

Subject map: Sciences

A degree in Sciences gives Students access to a large number of career choices. Many students who study sciences go on to pursue their Master's degree in Science. However, a significant portion of them also start looking out for jobs in the field of Cancer research, Stem Cell technology and other positions in this space.

Did you know? Being a scientist of any kind can open up may doors within any industries, from managing projects to labs to health policy teams with governments!



Find our about Science-related careers here:
PROSPECTS: https://www.prospects.ac.uk
TARGET JOBS: https://targetjobs.co.uk

For Teachers RBC Guide

Learner aims

The Research-Based Curriculum aims to support student attainment and university progression by providing classroom resources about cutting-edge research at local universities. The resources are designed to:

- ✓ promote intellectual curiosity through exposure to academic research
- ✓ stretch and challenge students to think deeply about content that may be beyond the confines of the curriculum
- ✓ develop core academic skills, including critical thinking, metacognition, and written and verbal communication
- ✓ inform students about how subjects are studied at university, and provide information, advice and guidance on pursuing subjects at undergraduate level

Content

The programme represents a unique collaboration between universities and schools. Trained by AccessEd, PhD Researchers use their subject expertise to create rich resources that help bring new discoveries and debates to students.

The Research-Based Curriculum offers ten modules suitable for either KS4 or KS5 study. The modules span a range of disciplines, including EBacc and A-level subjects, as well as degree subjects like biochemistry. Each module includes six hours of teaching content, supported by student packs, teacher notes and slides. All modules are available online and free of charge for teachers at select schools.

Using the RBC pack

These resources are designed to be used flexibly by teachers. The resources can be completed by students individually or in groups, in or out of the classroom.

For Teachers
Using the RBC packs

Extra-Curricular Subject Enrichment Clubs

Here are five examples of delivery options:

The resources can be completed in small groups (4-8 pupils) across a series of weekly lunch clubs or after-school clubs. Groups can reflect on their learning by presenting a talk or poster on the subject matter at the end of the course.

University Access Workshops

The resources can be used by students to explore subjects that they are interested in studying at university. This can inform their decision making with regards to university degree courses, and allow students to write more effective personal statements by including reflections on the Research-Based Curriculum.

Research Challenge

The resources can be used to ignite curiosity in new topics and encourage independent research. Schools could hold a research challenge across a class or year group to submit a piece of work based on the resources. Pupils could submit individually or in small groups, with a final celebration event.

Summer Project

Resource packs can function as 'transition' projects over the summer, serving as an introduction to the next level of study between KS3 and KS4, or KS4 and KS5. Students could present their reflections on the experience in a journal.

Why offer these?

The Research-Based Curricula programme builds on the University Learning in Schools programme (ULiS), which was successfully delivered and evaluated through the London Schools Excellence Fund in 2015. The project was designed in a collaboration between Achievement for All and The Brilliant Club, the latter of which is the sister organisation of AccessEd. ULiS resulted in the design and dissemination of 15 schemes of work based on PhD research for teachers and pupils at Key Stage 3. The project was evaluated by LKMCo. Overall, pupils made higher than expected progress and felt more engaged with the subject content. The full evaluation can be found here: ULiS Evaluation.

Questions

For more information contact hello@access-ed.ngo

Introduction to Topic Studying Mechanical Biotechnology at University

I did my life sciences undergraduate degree and Master's, both in Medical Biotechnology, at the University of Rostock in Germany. The bachelor degree is a 3-year course of 6 terms with module exams after each term.

It is a very interdisciplinary, research-driven degree offered by the medical school with input from the school of maths, sciences, informatics, electrical engineering and mechanical engineering. The modules taught range from science subjects such as chemistry, physics, and statistics over medical-related subjects such as anatomy, physiology, biochemistry, pathology, pharmacology to more applied or research-focused subjects such as bioethics, bio-law, molecular medicine and laboratory-only modules.

The course aims to provide knowledge and skills for fundamental as well as applied and clinical research, and contents is delivered in lectures, seminars, tutorials and lots of practical sessions in either teaching labs but also in a range of the university's research labs. Assessments are usually exams at the end of each term, but some modules require submission of reports, giving oral presentations or multiple in-class tests.

Additionally to the term-time teaching, in the teaching-free periods or term holidays the students are required to do a 6-week industrial placement as well as shorter lab placements in various research labs. The project work for the Bachelor's thesis is done over 9 weeks in the last term. As the degree itself is very broad and interdisciplinary, the options after the degree are varied too. Scientists with a degree in Medical Biotechnology are employed by universities, biotechnology companies, pharma industry, authority offices, consulting and insurance companies, patent agencies and many more.

Introduction to Topic Studying Mechanical Biotechnology at University

Areas of work can be fundamental research in any science field; medical research and development, drug discovery and development, quality control, lab-technicians, regulatory and administrative roles. The bachelor offers the foundations for a postgraduate study in a related field or the options to qualify further in subject-specific areas such as science writing, science or patent law, or economics.

As the degree itself is very broad and interdisciplinary, the options after the degree are varied too. Scientists with a degree in Medical Biotechnology are employed by universities, biotechnology companies, pharma industry, authority offices, consulting and insurance companies, patent agencies and many more. Areas of work can be fundamental research in any science field; medical research and development, drug discovery and development, quality control, lab-technicians, regulatory and administrative roles. The bachelor offers the foundations for a postgraduate study in a related field or the options to qualify further in subject-specific areas such as science writing, science or patent law, or economics.

Introduction to Research

My research project is interdisciplinary, looking at a set of polymer molecules that were developed for applications in drug delivery and their interactions with cell membranes. The intent is to understand which mechanisms are driving the interactions with a biomembrane and which factors can influence them, and to do this I use model membranes of different complexity in lipid composition to mimic cell membranes. During my studies I have used lipid monolayers and bilayers, as well as cancer cell lines and bacterial strains to understand the effects on the membranes.

The polymers in questions are available with different surface properties, which seem to cause different types of interactions with the membrane, but also show different levels of toxicity towards living cells. We need to understand the physical behaviour of the polymers in solution and which factors affect their behaviour and membrane interactions to be able to make improvements to the chemical composition of the polymers. The improved polymers structures can be then developed further as potential medicines. The knowledge coming from this fundamental research can also provide guidance, in which way the polymers will work best as a drug, i.e. tablet, patch, syrup etc; and for which disease they could be used as a therapeutic agent.

Meet the PhD Researcher Marleen Wilde

My academic history is not straight forward and probably not finished anytime soon either, but so far that has not been a problem, instead quite the opposite.

After leaving secondary school at 16 with a qualification similar to the GCSEs I went straight into a 3-year job qualification course in Medical-technical assistance for functional diagnostics. It is a German apprenticeship and covers diagnostics for heart and lung diseases, illnesses of the nervous system and brain, and diseases of the ear, nose and throat. I started working in the Cardiology department of the university hospital in Rostock, and after a year in the job I decided to do my A-level equivalent. For financial reasons I had to keep my job and go to evening college for this. After three hard years with not much free-time but growing knowledge in sciences, I came across a flyer at work advertising a new degree in Medical Biotechnology. Having already a medical background and a strong interest in science and research, I decided that was the degree to go for. Once I got my A-equivalent, I signed up for university and quit my job.

Admittedly, I found it hard to study and getting used to the university expectations. In school I never needed to study much at all as I understood and retained a lot during the lessons, so the need of being able to remember and recite vast amounts of information (i.e. biochemistry or anatomy) in the module exams was new to me. It took me nearly two years of my degree to learn how to study and to catch up with failed exams and the ever advancing difficulty level. I found the practical side of the degree very enjoyable and tried to work in as many different labs as possible to gain better insights into the research. My industrial/ non-university 6-week placement I did in Berlin in a cardiogenetic research lab. To support myself financially I also took on a part-time job as student research assistant in the department of Medical Physiology, where I also ended up doing my Bachelor's thesis studying epilepsy in rats.

Meet the PhD Researcher Marleen Wilde

Whilst I would have liked to get a research job straight after my degree, it turned out German employers were not used to 3-year university degrees yet as the system just recently changed from a normal 5-year program to the 3+2-years Bachelor and Master system. Hence, I came to the conclusion, that if I want a job, I need a master's degree and since it was convenient, I applied for the M.Sc. in Medical Biotechnology at Rostock.

During the master's I kept my part-time job as student research assistant, which not only provided money but also practical experience. I also took the opportunity to apply for an international, paid summer placement with the International Association for the Exchange of Students for Technical Experience. I ended up getting in space in Vienna (Austria) at Baxter Healthcare, where I was gaining experience in Quality Control techniques for medicines. Those 10 weeks in Vienna were inspiring. Whilst I came to the conclusion that Quality Control is not for me, I did enjoy the work in industry and having the intercultural exchange at work as well as with the other international placement students.

It did make me wanting to go abroad more, so after my third term of the master's before my thesis, I took a suspension to go to Italy to study Pharmaceutical Biotechnology for one term using the Erasmus agreement, which certainly broadened my horizon. As I was looking for place to do my master's project, the opportunity came up to come to England, to the University of Reading, again under the Erasmus scheme. So I ended up in a town I had never heard of before to do my master's project on membrane-interactions in the School of Pharmacy. After graduation back in Germany I got offered a position at Roche pharmaceuticals in Welwyn Garden City to work in clinical trials. I took it and really enjoyed the work environment and learning options, but after a while I got bored

Meet the PhD Researcher Marleen Wilde

of the routine and was looking to change. It was then I realised that all jobs I am potentially interested in, have the requirement of a PhD degree. Frustrated I decided to go back to university to do the doctoral research degree and was lucky to get a project with my supervisors from my master's. As my project is not funded by a research council or similar, I took on a teaching support role to finance myself and started undertaking the PhD degree part-time. I also have another smaller part-time job as an academic mentor for the disability service. By now I have done five years of my PhD and slowly coming to an end and writing up my work. For the time being, I do not know what comes after I finish, but I am sure it will be likely something completely different again.

A-Level Subjects	N/A
Undergraduate	Medical Biotechnology
Postgraduate	Master in Medical Biotechnology, PhD in Polymer membrane interactions surrounding drug delivery.

Glossary

Term	Definition
Acetyl	Acyl with chemical formula CH_3CO
Amide	Functional group containing a carbonyl group linked to a nitrogen atom
Amphiphilic	A chemical compound possessing both hydrophilic and lipophilic (fat-loving, hydrophobic) properties.
Antigens	Structures specifically bound by antibodies or antigen receptors and stimulating an immune response, specifically activating lymphocytes, which are the body's infection-fighting white blood cells
Bilayer	Double layer of closely packed atoms or molecules.
CL	Cardiolipin, net head charge -2
Compartmentalisation	Formation of cellular compartments within the cytosol of a eukaryotic cell, usually surrounded by a single or double lipid layer membrane
Endocytosis/ Exocytosis	Endocytosis is the process of capturing a substance or particle from outside the cell by engulfing it with the cell membrane, and bringing it into the cell. Exocytosis describes the process of intracellular vesicles fusing with the plasma membrane and releasing their contents to the outside of the cell
Ester	Functional group with the general formula RCOOR′, where R may be a hydrogen atom, an alkyl group, or an aryl group, and R′ may be an alkyl group or an aryl group but not a hydrogen atom.
Glycosylation	In Biology, it means the enzymatic process that attaches glycans (saccharide chains) to proteins, or other organic molecules.
Homogenous	Describes things that are all of the similar kind
Hydrophobic / hydrophilic	Hydrophobic: water hating Hydrophilic: water-loving (polar)

Glossary

Term	Definition
Lipid raft	Subdomains of the plasma membrane that contain high concentrations of cholesterol and sphingolipids
Moiety	Functional part or group of a molecule
Monolayer	A layer one molecule thick
Pathogen	Any microorganism that can cause a disease
Polarity	A separation of electric charge leading to a molecule or its chemical groups having an electric dipole moment, with a negatively charged end and a positively charged end.
PC	Phosphatidylcholine, head net charge 0
PE	Phosphatidylethanolamine, head net charge 0
PG	Phosphatidylglycerol, net head charge -1
PI	Phosphatidylinositol, net head charge up to -4
PS	Phosphatidylserine, net head charge -1
SM	Sphingomyelin, net head charge 0
Saccharides (Mono, Oligo, Poly)	Sugars, sugar-residuals connected to other molecules, mono = one unit, oligo 2 – 20 units, poly > 20 units
Staining / Counterstaining	Staining is a technique used to enhance contrast in samples, generally at the microscopic level. A counterstain is a stain with colour contrasting to the principal stain.
Toxicity	Degree to which a substance can harm humans or animals.

Resource One Overview

Topic	Lipids as building blocks
A-level Modules	Groups of lipids, saturated and unsaturated fatty acids, phospholipids
Objectives	By the end of this resource, you will be able to: ✓ Identify lipid building blocks and use them to identify and describe major lipid classes ✓ Relate the structure of lipids to specific functions within a membrane ✓ Consider the concept of lipid self-assembly and predict effect on the formation of a membrane
Instructions	1. read the source 2. answer the questions 3. explore the further reading 4. move on to the next resource

Resource One
Data Source

Section A

What are Lipids?

There are four main organic compounds found in organisms, namely proteins, carbohydrates, nucleic acids, and lipids. This resource is focussing on lipids, which in simple terms are fats, and can be found in the body in form of hormones or vitamins, but also form the major component of biological membranes. Membranes separate prokaryotes and eukaryotic cells and their organelles from the external environment.

Lipids in general are hydrophobic, and most of them are also amphiphilic, which means they carry both polar (i.e. headgroups) and non-polar (i.e. tails) domains. Like any other fats, they tend to be insoluble or poorly soluble in water; and therefore aggregate to reduce their contact with a watery (aqueous) environment. A real-life example would be cooking oil "swimming" on the water surface when you put both in the same pot.

Section B

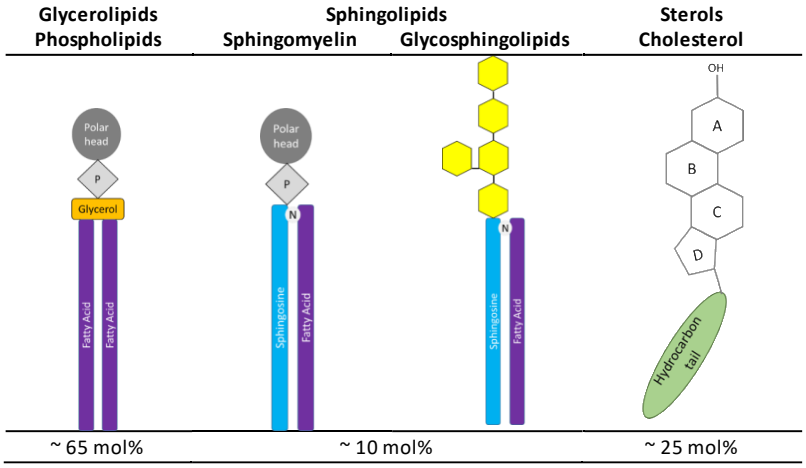
Lipid Classification

In the living organism, especially in relation to membrane lipids, we speak of three major groups of lipids according their chemical structure: steroids, glycero(phospho)lipids and sphingolipids. Within those classes there is a high chemical diversity, lipid studies suggest there are over 180 000 varieties in total. A simplistic illustration of the structural differences can be found in **table 1**. Fatty acids can be found as separate group too, but they also form part of the tail structure of lipids from the major groups. Some of the lipids of the major groups have carbohydrates attached to them, and therefore they are also referred to as glycolipids. A common glycolipid group are the sphingoglycolipids, which are discussed more in detail further below. There are also Phosphoglycolipids and glycophospholipids that contain both phosphate and carbohydrate as integral structural components.

Resource One
Data Source

Table 1

Overview of the three major membrane lipid classes and their simplified structures. %mol values indicate approximate percentage of the total of membrane lipids. P stands for phosphate group, N indicates the amide linker between the fatty acid and sphingosine.



Glycophospholipids have the structure of conventional phospholipids (phosphate group attached to glycerol linked with fatty acid chains), but instead having a typical headgroup attached to the phosphate, the moiety is glycosylated. An interesting example is phosphatidylglucoside which initially was only found in bacterial organisms, but over the last 20 years it has been detected in mammal cells, especially brain tissue, too.

Phosphoglycolipids on the other hand, are derived from glycosyldiacylglycerols, where the sugar group is attached to the glycerol binding to fatty acid chains). However, here there is not phosphate group between the glycerol and the sugar, but the sugar group itself is phosphorylated.

In plants glyceroglycolipids can be found which are composed of an acetylated or non-acetylated glycerol linked with at least one fatty acid. Those types of lipids often can be found in photosynthetic membranes and play a role in their functions.

Cholesterol can also occur glycosylated, a common example is cholesteryl glucoside which is mediator in signal transduction. Although sterol glycosides also have been found in plants membranes, especially in lipid rafts, little is known about their function.

Resource One
Data Source

Section C

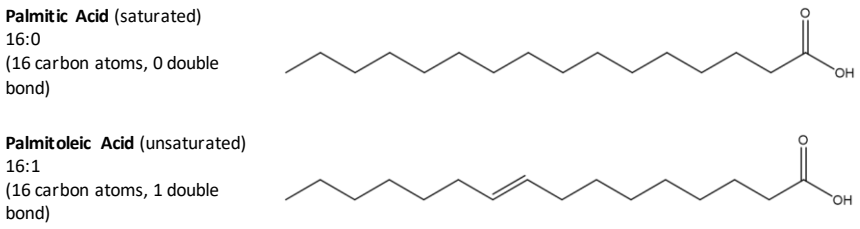
Fatty Acids

Fatty acids are hydrocarbon chains with a polar carboxyl group at one end (**figure 1**). Because this group ionises in water, fatty acids with short hydrocarbon chains (such as acetic acid) are actually well soluble in water. However, with increasing chain length, the water solubility and miscibility decrease and the fatty acids become of lipophilic (or in other words hydrophobic).

In organisms, the fatty acids mostly have linear hydrocarbon chains, with a common carbon atom number of 12-18. Eukaryotic organisms can express up to 26 carbon in chain length. Branched fatty acids are extremely rare. In general, mainly even numbers of carbon are found in organisms due to the fatty acid synthesis starting from a two-carbon compound. However, some bacteria express odd-numbered fatty acid chains.

Figure 1

Example of a saturated and unsaturated fatty acid with 16 hydrocarbon atoms, hydrogens are not shown. The carboxyl group is framed in red.



Both, saturated and unsaturated fatty acids can be found in biological membranes. Saturated fatty acids (an example is palmitic acid in **figure 1**) do not have double bonds, whereas unsaturated fatty acids have at least one double bond in the hydrocarbon chain. The double bonds cause bending of the chain, which makes formation of an orderly structure difficult.

Active reading

In summary, saturated fatty acids contain no carbon-to-carbon double bonds, monounsaturated fatty acids contain one carbon-to-carbon double bond, and polyunsaturated fatty acids contain two or more carbon-carbon double bonds.

Resource One
Data Source

Section D

Glycerophospholipids

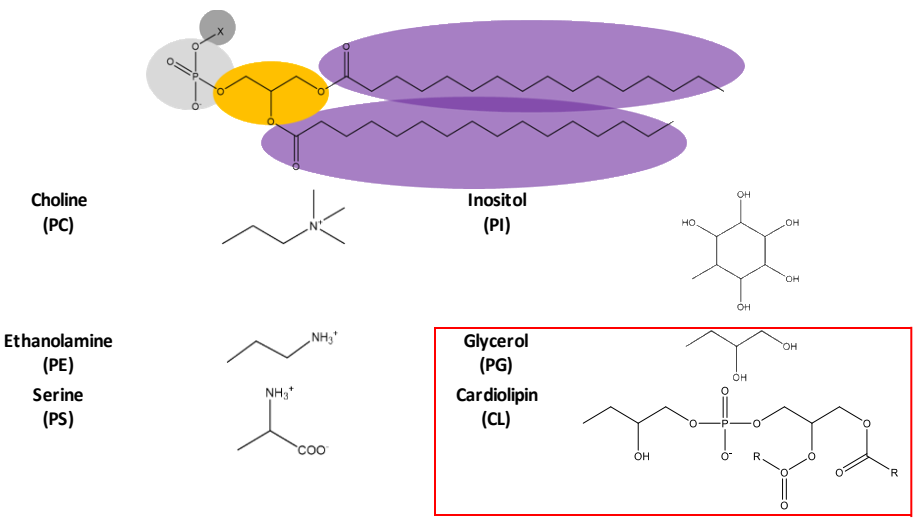
Glycerophospholipids or phospholipids present around 65 %mol of total membrane lipids of organisms and are the principal component of all cell membranes. There are usually composed of a polar headgroup, a negatively charged phosphate group, and a hydrophobic diacylglycerol (DAG) backbone (see **figure 2**). The DAG is a glyceride made of two fatty acid chains attached to a glycerol molecule by a covalent ester bond.

Active reading

Out of the headgroups in **figure 2**, there are two headgroups that result in a zwitterionic (electrostatically neutral) charge of the lipid, namely choline and ethanolamine. All other lipids carry a negative net charge at physiological pH of ~ 7.4. Four of the headgroups are commonly found in eukaryotic organisms, namely choline, serine, inositol and ethanolamine. There are also two lipids that are typical bacterial lipids (highlighted in the red box) and are usually not found in eukaryotic membranes. The exception is the inner mitochondrial membrane, of which ~ 25 % is made of cardiolipin, which supports the hypothesis that mitochondria can evolutionary traced back to bacteria.

Figure 2

General structure of a phospholipid and common headgroups (which replace the X in the illustration). The colours in the background break the structure down into its main components. The phosphate group is highlighted in light grey, glycerol in yellow, and fatty acid chains in purple. The headgroups highlighted in the red box are found in bacterial lipids.

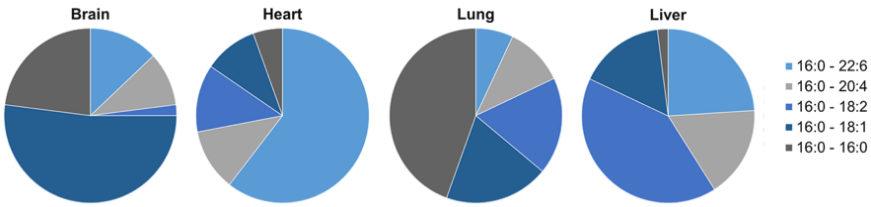


Resource One
Data Source

Phosphatidylcholine (PC) is a major zwitterionic membrane lipid that carries a negative charge from the phosphate group as well as a positive charge from the choline head. It is only abundant in eukaryotic organisms and provides the main structural support for the bilayer. It can be found with a wide variety of its fatty acid tails in terms of length and degree of saturation, and the distribution seems to be organ-dependent (figure 3).

Figure 3

Organ-dependent distribution of fatty acid chains of phosphatidylcholines (PC). To describe the lipid tail, the length and number of double bonds of each fatty acid is given. For example, 16:0 – 22:6 means, there is one saturated fatty acid with 16 carbons and 0 double bonds), and another polyunsaturated fatty acid chain with 22 carbons and 6 double bonds.



Phosphatidylethanolamine (PE) is another zwitterionic lipid, but with a smaller headgroup. It can be found both in eukaryotic and bacterial membranes and plays a role in membrane fusion processes. In fact, it is one of the major bacterial lipids aside phosphatidylglycerol and cardiolipin.

Phosphatidylserine (PS) is a lipid seen only in the cytosolic side (inner side) of the cell membrane and only in smaller quantities. It contributes to membrane asymmetry and has a function in cell signalling processes. The serine headgroup is has both an amine and a carboxyl group that contribute to the net charge of the lipid, but under physiological conditions the charge is -1 due to the phosphate group.

Phosphatidylinositol is another negatively charged lipid, carrying a neutral inositol residue, which plays an important role in cell signalling processes. However, it can only be found in low concentrations in the plasma membrane as can be seen in table 2.

Resource One
Data Source

Table 2

Phospholipid composition of plasma membrane and red blood cell membrane.

Phospholipid	Head Net Charge	Plasma Membrane	Red Blood Cell
PC	0	~ 45 %	~30 %
PE	0	~ 25 %	~28 %
PS	-1	< 10 %	~15 %
PI	up to -4	~ 5 %	< 1 %

Cardiolipin (CL) consists of two negatively charged phosphate groups that are linked to four unsaturated fatty acid tails via the glycerol groups. This results in a double negative charge of the lipid at physiological pH. CL is also seen as precursor molecule for phosphatidylglycerol.

Phosphatidylglycerol (PG) has only one phosphate group and a neutral glycerol headgroup and therefore, the lipid molecule carries a single negative charge. PG is a major lipid of bacterial membranes, but total percentage varies a lot between different types of bacteria as illustrated in table 3.

Table 3

Phospholipid composition of bacterial membranes. E. Coli and P. Aeruginosa are examples of gram-negative species, B. Subtilis and S. Aureus are gram-positive species.

Phospholipid	Head Net Charge	*Escherichia Coli*	*Pseudomonas Aeruginosa*	*Bacillus Subtilis*	*Staphylococcus Aureus*
PE	0	80 %	60 %	12 %	
PG	-1	20 %	22 %	70 %	58 %
CL	-2		11 %	4 %	42 %

Resource One
Data Source

Section E

Sphingolipids

Active reading

Sphingolipids are another major membrane lipid group of eukaryotic membranes, but they are not very common in membranes of bacteria or fungi. They are primarily found in the outer leaflet of the plasma membrane where they take part in cellular recognition, communication and signal transduction. Their polar headgroup is linked to the fatty acid and sphingosine component through an amide bond. Fatty acids chains of sphingolipids are usually saturated or mono-unsaturated with odd or even numbers of carbon atoms (up to 26). Sphingosine is an 18-carbon amino alcohol with an unsaturated hydrocarbon chain (top part of figure 4). According to their headgroup sphingolipids can be distinguished into the two bigger groups of phosphosphingolipids (i.e. phosphatidyl headgroup) and glycosphingolipids which have mono- or oligosaccharide groups attached (i.e. cerebrosides, gangliosides).

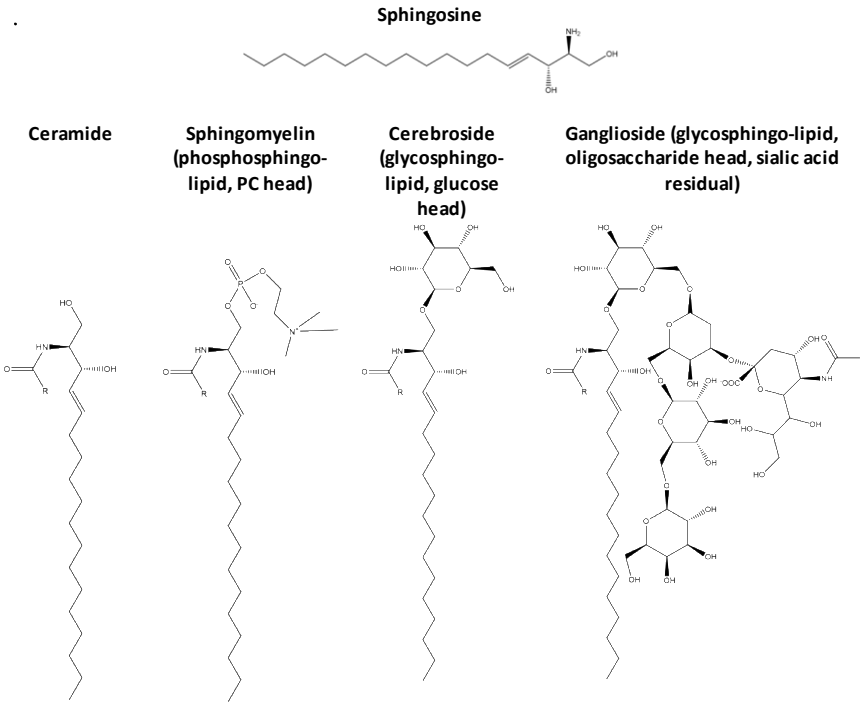


Figure 4

Diversity of Sphingolipids. Common features are the sphingosine backbone (see structure on the top) and the fatty acid tail (displayed as R in the chemical structure) connected to the head structure by an amide bond. A ceramide is the most basic sphingolipid. The dotted grey line indicates where the lipid headgroup including the amide bond ends and the lipid tail starts.

Resource One
Data Source

The most abundant species of the phosphosphingolipids is sphingomyelin (SM), a zwitterionic sphingolipid with a phosphatidylcholine headgroup. It usually constitutes 2–15% of the total organ phospholipid, but the tissues of the nervous system have an even higher amount of sphingomyelin in their membranes (especially in the myelin sheath surrounding the axons of neurons). Sphingomyelin, together with cholesterol, often forms lipid raft domains which are units of within a membrane responsible for specific cell functions.

The glycosphingolipids are fall also into the bigger group of glycolipids (any lipids with carbohydrate residues as their headgroup). One example are the oligosaccharide headgroups of the lipids of the red blood cell membrane defining the human AB0 blood types. Whilst the abundancy of glycosphingolipids is relatively low in mammalian and yeast plasma membranes, they have critical cell functions, mainly in cell recognition and signaling.

Section F
Sterols

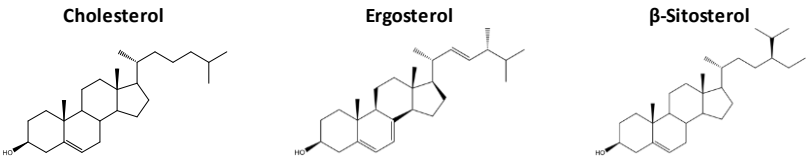
Sterols are the third big group of membrane lipids as shown in table 1. They also have an amphiphilic character due to the hydrophilic head region (hydroxyl group) attached to the hydrophobic tail (short hydrocarbon chain connected to four linked hydrocarbon rings).

In mammals, sterols are subdivided primarily according to their function: steroids, hormones and signalling molecules. Cholesterol (**figure 5**) and its derivatives are the most abundant lipids in animal tissue. Cholesterol itself makes up ~ 25 mol% of the total membrane lipids and can be found dispersed between the membrane phospholipids. It cannot assemble into bilayers on its own due its steroidal planar structure. The insertion of cholesterol prevents phospholipids of packing too tightly together and stops the membrane from becoming stiff. The highest amount of cholesterol is in the plasma membrane, but it can also be found in membranes of cellular organelles in varying quantities depending on the membrane function.

Resource One
Data Source

Figure 5

Chemical structures of the main sterol of animals (left), fungi and yeasts (middle) and plants (right).



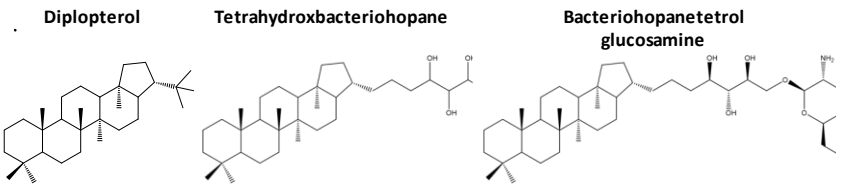
Membranes of plant cells do not have cholesterol, but so-called phytosterols such as ß-sitosterol (figure 5), stigmasterol or campesterol as their equivalent membrane lipids. Yeasts and fungi tend to have ergosterol as their main sterol.

Bacteria are believed not to express sterols at all. However, they have been found to express lipids alike to the eukaryotic sterols with similar functions, which are called hopanoids. Common examples are diplopterols and bacteriohopanepolyols (figure 6). The lipids are commonly located in the outer membranes of bacteria, where they intercalate into the lipid bilayers and affect the membrane fluidity and permeability.

The hopanoid lipids have four six-carbon rings and one five-carbon ring connected by one carbon-carbon single bond between two neighbouring rings, and they also contain different hydrophobic and hydrophilic side chains. Those various side chains contribute to the diversity of this lipid class and also account for the variety of functions of the bacterial lipids.

Figure 6

Chemical structures of common hopanoid lipids in bacteria.



Resource One
Data Source

Section G

Lipid self-assembly

Membrane lipid molecules are amphiphilic having a hydrophilic headgroup and hydrophobic tails. This means, when they are placed in water or another aqueous medium, they assemble spontaneously into bilayers. If their molecular shape allows it, their hydrophobic tails will face away from the aqueous medium, whereas the hydrophilic head will face towards it.

Lipids, especially phospholipids, are very diverse in their chemical composition and therefore also differ in their molecular shape. In general, most of the phospholipids fit in one of three distinguished geometries: conical, cylindrical or inverse conical, which are determined by the size of the headgroup in relation to the lipid tail(s).

The geometrical shape of the lipids defines the membrane structures they would form after self-assembly (**table 4**). For example, PE has a conical shape and therefore would form a negative membrane curvature if many lipid molecules assemble together, to the extent that they could form spherical structures called micelles. Other cylindrical lipids such as PC would always form a flat bilayer sheet without curvature.

In reality, a biological membrane is formed by a mix of different lipids with different geometry, which is necessary for the cell curvature, to accommodate proteins of different shapes and to facilitate cell processes such as endo- and exocytosis.

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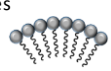
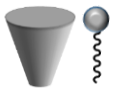
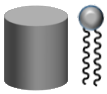
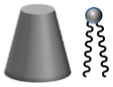
Resource One
Data Source

Table 4

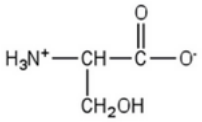
Impact of molecular shape on lipid self-assembly.

Lipids	Membrane curvature and Phase behaviour	Molecular Shape
Large headgroup, acyl chain(s) occupy smaller volume Lysophospholipids, Phosphoinositides (PIP_2, PIP_3). Detergents	☐ Positive membrane curvature ☐ Favour the assembly membranes into normal micelles or cubic structures 	Inverted Conical 
Diameter of headgroup and acyl chains similar SM, PC, PS, PI, PG Phosphatidic Acid	☐ No membrane curvature ☐ Favour the assembly into lamellar structures or flat bilayers 	Cylindrical 
Small headgroup, acyl chains occupy large volume PE, PS at pH < 4, CL Phosphatidic Acid at pH < 3	☐ Negative membrane curvature ☐ Favour assembly into tubular/hexagonal or spherical inverted micelles 	Conical 

Resource One Activities

Activities

1. Serine is an amino acid with following structure:



Draw the structure for a phosphatidylserine, which contains a palmitic acid and a palmitoleic acid unit in the tail.

2. Name the structural unit that must be present for a molecule to be classified as a
   a) phospholipid.
   b) glycolipid.
   c) sphingolipid.

3. In cerebrosides, what is the linkage (the chemical bond) between a fatty acid and sphingosine?

4. Why is it important that membrane lipids have dual character—part of the molecule is hydrophilic and part of the molecule is hydrophobic?

Problem solving

5. Identify a key function for each Lipid
   a) Cholesterol
   b) Sphingomyelin
   c) Phosphatidylinositol
6. Discuss the difference between conical and inverted conical lipids.
7. Summarise the structure of a fatty acid and explain saturated, monounsaturated and polyunsaturated.

Resource One
Further Reading

Explore

- https://www.youtube.com/watch?v=H44rdXEVXGg Phospholipids Structure and Function – Revision for A-levels
- https://jcs.biologists.org/content/124/1/5 - Review Article and poster of Lipid Map of the Mammalian Cell including poster with illustrations of Lipid Synthesis Pathways and Lipid Distribution in different Membranes
- https://www.lipidmaps.org/resources/databases/index.php?tab=lmsd Largest public lipid database in the world.

Resource Two Overview

Topic	Lipid Membranes of Bacterial and Eukaryotic Cells
A-level Modules	Structure of eukaryotic cells related to cell-surface membrane/ cell wall, membranes of cell organelles Structure of prokaryotic cells related to cell wall; glycoproteins Structure and function of biomembranes, fluid mosaic model
Objectives	By the end of this resource, you will be able to: ✓ Describe and compare typical components of membranes of bacterial and eukaryotic organisms. ✓ Discuss the Fluid Mosaic Model and factors influencing the structural make-up of a cell.
Instructions	1. read the source 2. answer the questions 3. explore the further reading 4. move on to the next resource

Resource Two
Data Source

Section A

Biological Membranes

Membranes form one of the key structures of living cells, and this resource is going to focus on membranes of bacterial (eubacteria) and eukaryotic organisms only. Membranes of Archaea will not be addressed due to the complexity and diversity of their properties.

All cells have an outer plasma membrane, but unlike bacteria, eukaryotic cells also possess internal membranes that surround their organelles. In general, all biological membranes are bilayers (two planar leaflets) composed of lipids, proteins, and carbohydrates, whereby the protein/lipid ratio can differ from 1:4 to 4:1 depending on the membrane type and its function. Carbohydrate moieties are always located on the outside of the membrane in the outer leaflet and the overall thickness of membranes can vary from 40 – 100 Å.

General membrane functions are:

- Protective barrier, selectively permeable
- Transport of molecules via channels, gates and pumps
- Metabolism (Endocytosis, exocytosis)
- Receptors for outside signal detection: light, odour, taste, chemicals (hormones, neurotransmitters, drugs)
- Cell signalling processes (intracellular and extracellular)
- Docking station for proteins, cytoskeleton
- Compartmentalisation (i.e. cell organelles)
- Cell polarisation (electric and chemical potential): inside negative ~-60 mV
- Energy Transduction: photosynthesis, oxidative phosphorylation

Resource Two
Data Source

Section B

The Fluid Mosaic Model

The "Fluid mosaic model" is a theoretical model of the structural arrangement and molecular composition of (eukaryotic) cellular membranes originally proposed by Singer & Nicolson in 1972. In this model, the bilayer is formed by self-assembly of lipids and is considered to be fluid as its components (lipids, membrane proteins) can move sideways along the membrane or even across the leaflets. Thus, the arrangement is a dynamic process that it is continuously changing the "mosaic pattern". That means the membrane itself is not solid and static but behaves more like a viscous liquid or "fluid". The model has been adapted over time to accommodate new information from research on different membrane components (i.e. lipids, proteins, saccharides) and membrane arrangements and functions (i.e. lipid rafts, protein interactions).

Section C

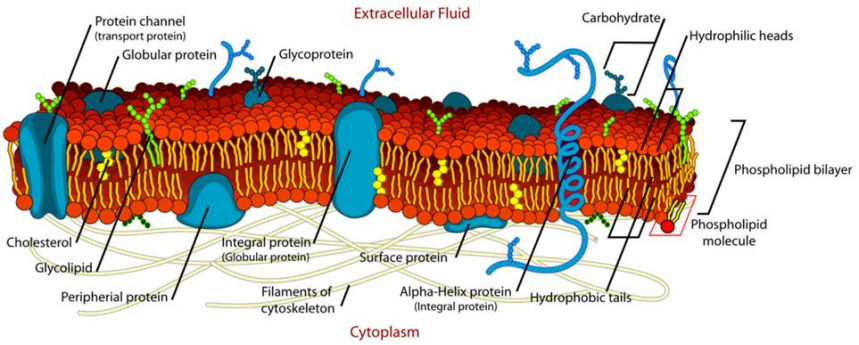
Basic Structure and Components of a cell membrane

The plasma membrane of cells is asymmetric in terms of surface ion composition and distribution of proteins and lipids (**figure 7**). The proteins present in a particular membrane regulate the substances to which it will be permeable and what signal molecules it can recognise. The lipid asymmetry is also maintained by lipid translocation or transport proteins which add to the diversity and complexity of cellular compartments. Extracellular parts of lipids and proteins are frequently glycosylated. Glycans form a dense structure at the outer surface of the plasma membrane and are important for cell recognition and signalling processes.

Resource Two
Data Source

Figure 7

Schematics of a cellular membrane according to the fluid mosaic model.)



Picture by LadyofHats Mariana Ruiz - Own work. Image renamed from File:Cell membrane detailed diagram.svg, Public Domain, https://commons.wikimedia.org/w/index.php?curid=6027169)

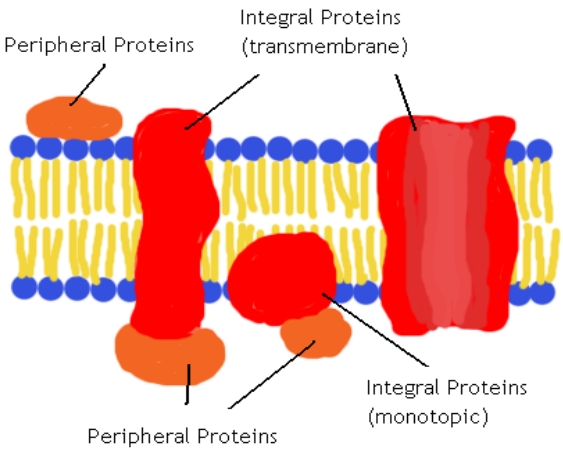
Lipids, as one of the major components of a membrane, form a semi-permeable lipid bilayer. This a lamellar structure with a hydrophobic core and a polar headgroup region on both sides cells and it is composed of hundreds of different phospholipid species. These differ in their polar headgroup moiety but also in the length and saturation of acyl chains of the tails forming the hydrophobic core of a lipid bilayer. Other lipid and fatty acid species add to this complexity. Of those, sterols (cholesterol in mammals) are the most abundant in the plasma membrane and can represent up to 40% of total lipid. Cholesterol has a planar structure driving its strong impact on basic membrane properties such as viscosity or fluidity. The structural organisation of the bilayer can be explained with the Fluid Mosaic Model.

Proteins make up ~ half of the total plasma membrane mass and their principal task is to help the cell to function by sending and receiving messages and transporting molecules across cell and compartmental membranes. Membrane proteins are required by both unicellular and multicellular organisms to live and survive, and they can be distinguished into integral and peripheral membrane proteins based on their association with the lipid layer.

Resource Two Data Source

Figure 8

Simplified overview of types of membrane proteins and their location within the membrane.



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Integral membrane proteins are permanently embedded within the bilayer and can be further differentiated into transmembrane proteins (span the entire membrane) or monotopic proteins (only inserted in one leaflet of bilayer).

Peripheral membrane proteins are only temporarily attached to either integral membrane proteins or the membrane lipids. They are mostly hydrophilic and can be easily removed, which is useful for cell signalling processes. Therefore, they are often associated with ion channels and transmembrane receptors.

Different proteins have different functions:

- Structural proteins support the cellular shape
- Receptor proteins help communication and signalling, i.e. by using hormones or neurotransmitters
- Transport proteins (i.e. globular proteins, channel proteins) facilitate diffusion of molecules across the membrane
- Glycoproteins aid cell communications and transport of molecules across the membrane through their attached sugar moieties

Resource Two
Data Source

Section D

Asymmetry of a membrane – Distribution of lipids

Active reading

The distribution of lipids between the inner and outer leaflet of membrane is not equal and therefore has important physiological implication on the functions of a membrane. The unequal lipid distribution produces electric charge gradients across the bilayer, within the same leaflet as well as across the leaflets. As an example, table 5 illustrates the lipid asymmetry of the human red blood cell. In general, the cytosolic inner membrane is more negatively charged since negatively charged lipids such as phosphatidylserine (PS) and phosphatidylinositol (PI) are primarily found in the inner leaflet. The outer leaflet predominantly contains neutral lipids such as phosphatidylcholine (PC) and sphingomyelin (SM), whereas neutral phosphatidylethanolamine (PE) is primarily found in the inner leaflet.

Table 5

Asymmetry in phospholipid composition of a human red blood cell (Erythrocyte).

Phospholipid	Inner leaflet	Outer leaflet
PC and SM	~ 23 %	~ 89 %
PE	~ 44 %	~ 11 %
PS, PI, Phosphatidic acid	~ 33 %	0 %

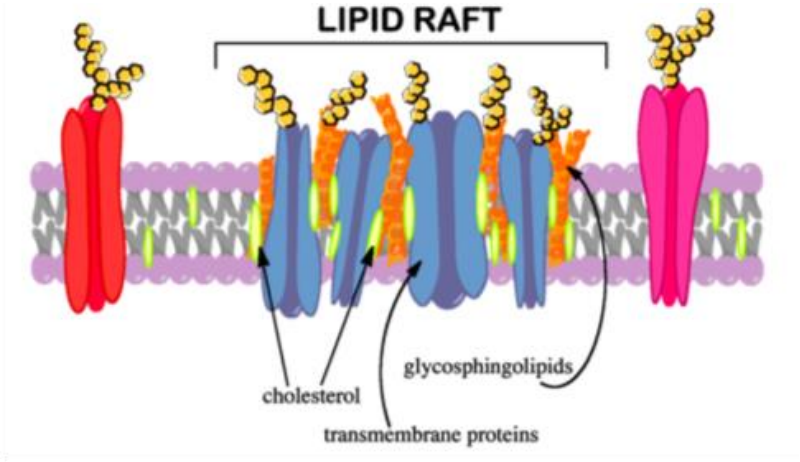
Furthermore, glycolipids such as sphingoglycolipids are exclusively found in the outer leaflet, where they fulfil important cell functions such as signalling and recognition, communication, and maintaining a hydration layer close to the membrane interface.

Cholesterol is not evenly distributed in the membrane either, but often found with sphingolipids or certain phospholipids, where it inserts its backbone close to the lipid chains and thereby forms condensed microdomains called lipid rafts (figure 9). Those rafts tend to be more rigid then the rest of the surrounding bilayer and are important for lipid-protein interactions as they compartmentalise functional proteins. They also serve as anchor points for transmembrane or peripheral membrane proteins and thereby they support cellular processes, regulatory and signalling events.

Resource Two
Data Source

Figure 9

Illustration of a lipid raft.



By Lizanne Koch lgkoch - own work with ChemBioDraw, Public Domain, https://commons.wikimedia.org/w/index.php?curid=61799352

Section E

Eukaryotic versus bacterial cells – What's the difference?

Whilst not discussed in detail, eukaryotic cells are much bigger in size than bacteria and also use membranes to compartmentalise their functional organelles.

In terms of membrane structure, the basic bacterial cell membrane differs quite a bit from that of eukaryotic cells and those structural differences also result in a much higher thickness of the protective cell envelope.

Active reading

In general, bacteria can be distinguished into gram-negative and gram-positive species which is depending on the structural configuration of the cell envelope (**figure 10**). The cell wall thickness of gram-negative bacteria varies between 2-3 nm, the one of gram-positive bacteria between 20-80 nm.

The lipid bilayer as cytoplasmic membrane is existent in all species; however, bacteria have a thicker cell envelope overall mainly due to the peptidoglycan layer which functions as another protective layer. Gram-negative bacteria have a thinner peptidoglycan layer than gram-positive bacteria but have yet another protective layer atop of it – the outer membrane. The peptidoglycan layer (and outer membrane) is also referred to as cell wall.

Resource Two
Data Source

Figure 10

Schematic illustration of the cell envelope of bacterial cells .

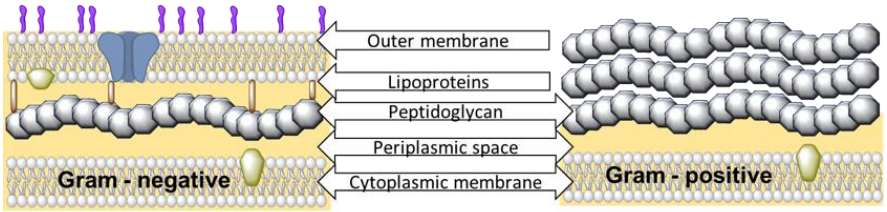
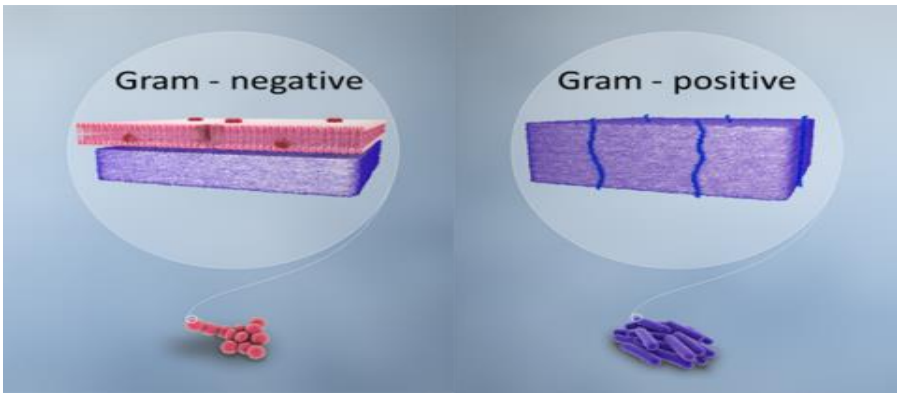


Figure 11

. Principle of Gram-staining of gram-negative and gram-positive bacteria. Crystal violet dye stains the peptidoglycan layer of the cell wall blue.



Adapted from https://ccsearch.creativecommons.org/photos/d324385a-de76-4344-aa2d-99448adcd2f0

Section E

Eukaryotic versus bacterial cells – What's the difference?

Gram stain, aimed at the bacterial cell wall, is commonly used for general bacterial identification. Gram-positive bacteria turn blue as they retain the crystal violet dye even after counterstaining with red safranin; whereas gram-negative bacteria will show a red colour as their cell walls do not retain the violet dye (**figure 11**).

Examples of gram-negative pathogens are Escherichia coli, Salmonella species, Pseudomonas aeruginosa, Legionella species, Klebsiella species, and Neisseria gonorrhoea. Gram-positive examples are Staphylococcus aureus, Streptococcus pneumonia, Clostridium botulinum, and Bacillus anthracis. They all can cause different types of infections, and different types of antibiotics are effective against them.

Not all bacteria are pathogens or cause infections. There are "good" bacteria in the gut, e.g. Bifidobacteria (gram-positive) or Enterobacteriaceae species (gram-negative).

Active reading

Resource Two Data Source

Others than the structural difference in their membranes, there is also a difference in the lipid composition of bacterial and eukaryotic membranes. Phosphatidylcholines (PC) are zwitterionic major eukaryotic membrane lipids that are rarely found in bacterial species, whereas anionic phosphatidylglycerols (PG) and cardiolipin (CL) represent almost exclusively bacterial lipid species. Looking at the lipid headgroup charge the anionic lipids in eukaryotic cells (including cancer cell lines) only make up a total of ~ 20 % of the membrane phospholipids (table 6). In comparison, depending on species, anionic phospholipids contribute up to 100 % to the net charge of the bacterial membrane (table 7).

Table 6

Phospholipid composition of examples of cell membranes in humans.

Phospholipid	Head Net charge	Plasma membrane	Red blood cell	Cancer cell line MCF7	Cancer cell line MDA-MB-231
PC	0	~ 45 %	~30 %	50 – 54 %	43 – 45 %
PE	0	~ 25 %	~28 %	18 – 20 %	10 – 18 %
PS	-1	< 10 %	~15 %	~ 6 %	6 – 10 %
PI	up to -4	~ 5 %	< 1 %	5 – 10 %	< 10 %

Table 7

Phospholipid composition of cell membranes of example bacteria.

Phospholipid	Head Net Charge	*Escherichia Coli*	*Pseudomonas Aeruginosa*	*Bacillus Subtilis*	*Staphylococcus Aureus*
PE	0	80 %	60 %	12 %	
PG	-1	20 %	22 %	70 %	58 %
CL	-2		11 %	4 %	42 %

Furthermore, membranes of bacteria do not contain sterols such as cholesterol, but instead hopanoid lipids which resemble steroids in structure. However, their function in membranes is different from steroids, since hopanoids decrease the fluidity and permeability of membranes. As the hydrophobic part of the lipids inserts between the phospholipid tails, it fills the empty spaces to an extend that it increases membrane integrity and stability. Meanwhile, the hydrophilic side chains can interact strongly with its environment, packing the membrane molecules tighter and reduce molecule permeation.

Resource Two
Data Source

Lipopolysaccharides are a major component of only bacterial cell membranes and can be found outer membrane leaflet. Those complex lipid-sugar formations are main contributors to the bacterial pathogenicity and toxicity.

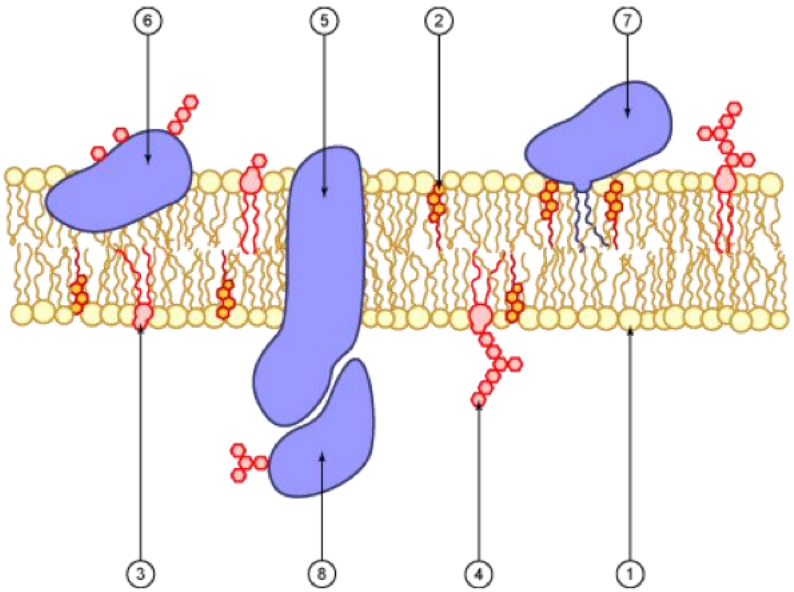
Additionally, bacteria can produce phosphorus-free membrane lipids such as ornithine lipids or sulfolipids, which contribute to the bacterial species diversity.

Resource Two Activities

Activities

Problem solving

1. Name four general membrane functions
2. Distinguish between an integral protein and a peripheral protein.
3. What are the functions of glycoproteins?
4. Relate the principles of the fluid mosaic model to the structural arrangement of a eukaryotic cell.
5. Annotate the numbers of following illustration:



6. Discuss the differences and common features of Escherichia Coli and Staphylococcus Aureus.

Resource Two
Further Reading

Explore

- https://www.nature.com/scitable/topicpage/cell-membranes-14052567/

- https://youtu.be/BpBzMh5QnHw - Centre for membrane interactions and dynamics

Resource Three Overview

Topic	Modelling – Not only for the catwalk
A-level module	Methods of studying cells Functions of and structure of membranes Considering factors affecting absorption of molecules
Objectives	After completing this resource, you should be able to: ✓ Differentiate between membrane models and model membranes and discuss their suitability and applications ✓ Categorise the different approaches of model membranes and outline the most common ones. ✓ Elaborate on parameters that can be controlled in experimental conditions.
Instructions	1. read the source 2. answer the questions 3. explore the further reading 4. move on to the next resource

Resource Three Data Source

Section A

What are membrane models and model membranes?

When we propose ideas how a membrane might be structured, what its components are, which interactions or processes take place between them, then we talk of membrane models in a theoretical sense. A widely adopted membrane model is the Fluid Mosaic Model. But there have been other models before that, and since then knowledge has evolved and the models were adapted.

As knowledge is deriving from research to update the model, both the membrane models in a theoretical context and the model membranes used in membrane-related research go hand in hand and rely on each other.

Active reading

In a practical sense, a model membrane is a simplified representation of a biological membrane and there are many different degrees of complexity available depending on the research question to answer.

Model membranes are used to study biological membrane components and mechanics, membrane interactions and their influencing factors, membrane permeability, biochemical pathways, activity and uptake of new medicines and more. Some of the biomedical research is focused on lipids only, other includes membrane components such as proteins and carbohydrates. Three approaches to study membranes can be distinguished: biophysical, biological and computational methods. They all have advantages and disadvantages) but are complementary to each other and can provide useful information.

Resource Three
Data Source

Section B

Biophysical model membranes

Many physical techniques are usually used for non-living matter are often not suitable for living cells. To overcome this issue, the field of biophysics emerged and with it the use of very simplified model membranes (table 8).

Those basic model membranes can made of a single lipid variety or a mixture of multiple lipids or a mixed lipid extract of a dead cell. To add complexity, proteins and receptors are added sometimes. Many of those lipid or protein components are commercially available, can be synthesised if the desired structure is known or can be extracted from cells.

Active reading

There are a number of biophysical different techniques that can be used with the model membranes. The most common ones are microscopic and spectroscopic techniques or a combination of both types (see paragraph in further reading section).

Table 8

Common biophysical model membranes.

	Monolayers 	Supported Bilayers 	Liposomes/ Vesicles 
Controllable parameters	- lipid composition - lateral pressure	- lipid composition - incorporation of integral proteins/ compounds - membrane curvature/ patterning	- lipid composition - membrane curvature/ lipid packing - incorporation of proteins - membrane deformation
Major advantages	- defined geometry of lipid assembly - homogenous system - individual components can be studied	- flat geometry - asymmetric lipid distribution possible - accessibility of both leaflets - compatible with multiple methods	- simple preparation - free-standing membrane - suitable for microscopic techniques due to micrometre-scale - compatible with multiple methods
Major disadvantages	- non-physiological single leaflet presentation - restricted to planar lipid monolayers (and mainly cylindrically shaped lipids)	- interactions with solid support cause restricted fluidity of lipids and non-physiological lipid distribution between the leaflets - possible defects within a bilayer	- possible variability in terms of size and multilamellarity - potentially size below optical resolution - only outer leaflet accessible - no control of lipid distribution in a mix

Resource Three Data Source

Section C

Basic biological techniques and model membranes

Biological experiments can be done either in vitro or in in vivo. Both words derive from Latin, "in vitro" means within the glass, in vivo stands for "within the living".

In vitro techniques are performed within a in a controlled environment outside of a living organism or their biological context and are explored further in detail below. This includes techniques in cellular biology or microbiology as well as using partial or dead organisms. One of the weaknesses of these techniques is the isolation from their natural living conditions and therefore a change in their functional behaviour.

In vivo studies perform experiments on whole, living organism; and animal studies and clinical trials are two forms of this research type. The advantage of this type of studies is the overall effects on a living subject can be observed. However, the information gained from animal experiments cannot always be translated into a human context because of interspecies differences.

In vitro techniques

Two simplified models are (eukaryotic) cell culture and microbiological culture, but there are also other more complex types that involve culturing tissues or organs. Cells and microbes can be grown as single population but also as more complex mixed systems to reflect more natural conditions.

Culturing is a process, by which cells (or organs or organisms) are grown and maintained under controlled laboratory conditions. These conditions vary depending on what is cultured, but normally involve environmental conditions (temperature, pH), type of growth vessel (culture flasks, petri dishes etc.), type of growth medium or substrate, and the supply of nutrients, growth factors, hormones and gases (CO_2/O_2). Cells can be cultured attached to the vessel or free floating (= suspension culture).

Resource Three Data Source

Cell culture

All cells start as a single cell isolate from a living tissue and are then maintained under controlled and optimised conditions as a culture (a population of cells with the same genetic makeup), which then is used for further testing. In general, one can distinguish between primary culture and immortal cell line.

Primary cells are extracted and cultured directly from a living tissue and tend to have a certain lifespan which determines how often the cells multiply until they either die or mutate. An example of primary cells is Human Umbilical Vein Endothelial Cells (HUVEC), which are used as a model for a broad range of research question and are isolated from the vein of the umbilical cord of human donors.

Immortal cell lines are also commercially available and are cells that either are genetically transformed to multiply indefinitely or are naturally immortal, which is true for many cancer cell lines. The first and most famous immortal cell line is HeLa, which derived from cervical cancer cells (further reading). Other well researched cancer cell lines are MCF7 or MDA-MB-231, both breast cancer cell lines.

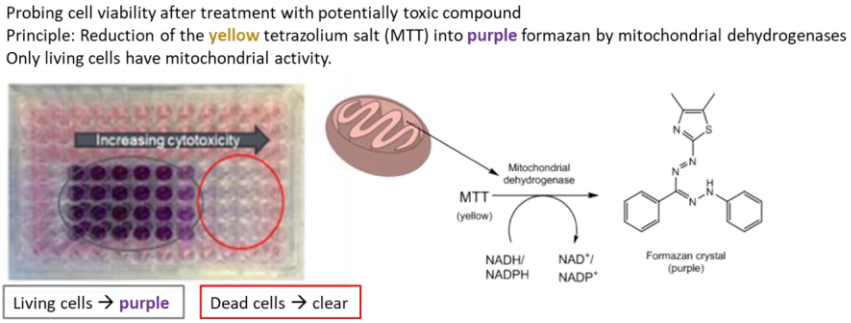
In the area of membrane interactions, cellular uptake of compounds through cell membranes, cell toxicity (**figure 12**), lipid up- or down-regulation, and much more can be studied on cultured cells.

Overall, there are many different cell lines available from cell banks, which can be studied for different purposes, but not all of them are human cell lines. The American Type Culture Collection alone offers over 3,600 cell lines from over 150 different species.

Resource Three Data Source

Figure 12

Cell toxicity evaluation on cell lines using the MTT test. A colorimetric assay is used to read the absorbance values of the 96 well plates, which can be normalised and the percentage of living cells can be calculated.



Microbiological culture methods

Microbiology methods are an important tool in biomedical research, especially when studying infectious diseases or the activity of new antimicrobial drugs. Bacteria, fungi, and yeasts are the common organisms studied in this field and in comparison, to the previously discussed eukaryotic cell lines, they are in general more robust and easier to culture. Because of their robustness, the effect of changing their environmental conditions such as pH of the growth medium or the temperature can be studied too. In fact, research suggests that those organisms respond to external stress with changes in their membrane composition and up- and down-regulation of certain lipids.

Microbes are either growing on solid media, such as agar plates (**figure 13**), or in liquid suspension and are easier to keep as they multiply much quicker than eukaryotic cells. The strains can be either commercially obtained or directly isolated from clinical samples (or other substrates such as soil for non-biomedical research). Theoretically, any microbe can be put on culture as long as the right living conditions are provided.

Resource Three Data Source

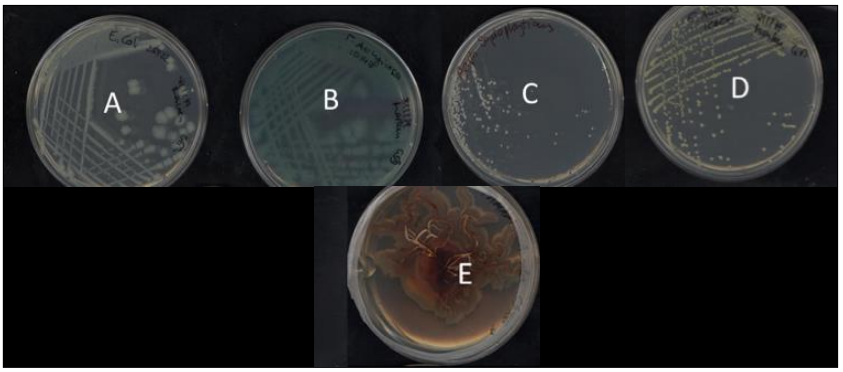


Figure 13:

Petri dishes with bacterial and fungal cultures. A) Escherichia Coli; B) Pseudomas Aeruginosa; C) Staphylococcus Saprophyticus; D) Staphylococcus Aureus; E) Armillaria fungus. As can be seen in the picture, bacteria have very distinct growth colonies, which can differ in colour, size and shape which is also useful in order to identify and distinguish them. Just by looking at the cultures, contamination with other strains are easily spotted. One single colony can contain millions of individual bacterial cells, all having the same genome and originating from a single cell. For example, Escherichia coli divides every 30 min and it takes only 10 hours to have more than 1 million bacteria deriving from a single one.

Similar to eukaryotic cell culture, microbial cultures can be used to study membrane-related topics, but also to explore new antimicrobial medicines. As the genome of microbes is comparatively small, the genome of the cultures is often sequenced to understand genetically driven adaptation processes, and therefore they are a useful model system.

Resource Three Data Source

Section D

An introduction to computational techniques and model membranes

Active reading

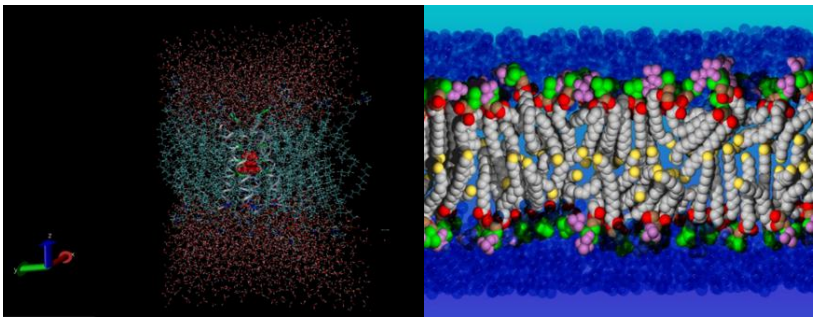
Computational studies are also referred to as in silico, a pseudo-Latin term for "in silicon", a material that is used a lot for computer chips. Albeit a fairly new field of research, it is continuously growing and has been adapted widely to predict how medicines interact with the body and with pathogens. Computational techniques can use the most basic model membrane to complex whole cell simulations. However, there are limitations to this approach based on the computer power needed and also the existing knowledge of biological systems and processes that still has major gaps. Practically speaking, only known factors can be set up as parameters in computational model, but not the unknowns that are present in an in vitro or in vivo experiment. Computational methods, also called molecular modelling, are used to model or mimic the behaviour of molecules. Common applications are computational chemistry, drug design or computational biology to study molecular systems ranging from small chemical systems to large biological molecules. Most molecular membrane modelling methods are either molecular dynamics or coarse-grained simulations (figure 14). Other types of molecular modelling are so called molecular docking studies (focused on binding of two molecules, i.e. drug and receptor), bacterial sequencing techniques and bacterial whole cell simulations. Molecular dynamics (MD) is a method for analysing the physical movements of atoms and molecules and is therefore also called an atomistic simulation. This means every individual atom in the system is accounted for in the simulation. The atoms and molecules are allowed to interact for a fixed period of time and therefore adding a dynamic dimension to the system. Because biological molecular systems usually consist of a vast number of particles, there are limitations on the size of a system that can be modelled this way. The bigger and more complex the simulated system is, the more difficult it is to avoid cumulative errors produced by the mathematical algorithms and parameters of the simulation.

Resource Three Data Source

Figure 14

Left: Snapshot of an atomistic MD simulation of a channel transmembrane protein embedded in a lipid bilayer with a drug inside blocking the channel. Outside the lipid bilayer is bulk water

. Right: Snapshot of a DPPC simulation using the coarse-grained approach. PO4 - green, N(CH3)3 - violet, water - blue, terminal CH3 - yellow, O - red, glycol C - brown, chain C - grey.



Coarse-grained models try to simulate the behaviour of complex systems using a simplified (coarse-grained) representation of the molecules. It is often used to study biological molecules such as proteins, nucleic acids, lipid membranes and carbohydrates, but also interaction of those with other molecules. This type of simulation can be set up at various granularity levels, which means using "pseudo-atoms" representing groups of atoms (i.e. as whole amino acid residue). By using this approach much longer simulation times and bigger systems can be studied, but at the expense of losing atomistic details. Thus, this method is sometimes combined with the molecular dynamics technique.

Section E

Controlling experimental conditions

Active reading

No matter which approach is taken to model membranes or to make membrane models, it is important to control the experimental conditions and fix the variables in the setup.

Typically, there are three major categories that need to be addressed as they affect the biological system of interest: solvent, temperature and pH.

Resource Three Data Source

Temperature

Biological experiments usually take place under in incubators under physiological temperature conditions, most eukaryotic cells are not able to survive long outside the body temperature range (37°C). Bacterial cells have different optimum temperatures but are quite forgiving and can survive in a wider temperature range. In microbiology experiments, bacteria, especially human pathogens, tend to be kept at body temperature too in order to study them in a medically relevant environment.

Biophysical experiments on the other hand, are often done at room temperature, either because the instrumental setup does not allow for temperature control or the model membrane is so simplistic that temperature will have no or only little effect on it. Nonetheless, it is acknowledged that temperature is an important factor and can impact on lipid fluidity or protein folding.

With computational methods, it is possible to include a fixed temperature to the system studied, although it is not always done. However, simulations are a useful to predict a temperature effect which cannot always be studied appropriately in a biological experiment.

pH

Another important parameter is the pH, here we need to think of the physiological cytosolic pH (~7.4), as well as different pH environments of organs (i.e. stomach ~1.5 – 3.5), cell organelles (i.e. lysosomes ~4 – 5)) and the pH of the surrounding medium, which can be controlled experimentally.

Again, living cells have a small pH tolerance range, and therefore are kept in a growth medium adjusted to the physiological pH. This makes it difficult to study the effect of a changing pH environment. It can be studied to a certain extend in bacterial culture, but there are limits to it too.

Resource Three Data Source

Conversely, biophysical methods are very well suited to explore membranes under different solvent pH conditions, as lipids itself are stable over a wide pH range. Therefore, by varying the lipid composition to mimic different type of membranes, including those of cell organelles, a wide range of membrane-related questions can be investigated.

pH parameters can also be set indirectly in computational simulations by adjusting the theoretical charge of molecules, which is dependent on the pH. However, pH cannot directly be defined with the ions present under biophysical pH conditions.

Solvent

The solvent conditions are also vital to the experimental setup and are the most controllable of all three parameters. In general a solvent can be defined by type (i.e. phosphate buffer, methanol), purity (important for chemical solvents) and if it is a mixture (i.e. growth medium). Sometimes residuals of chemical solvents in phosphate buffer or growth medium can affect the experiments too.

In biological experiments, the solvent is usually the optimised growth medium for the organisms in culture, sometimes with addition of specific nutrients or growth hormones.

The solvents used for biophysical studies can range from the more physiological phosphate buffer to non-physiological chemical solvents such as methanol, depending on the technique used and the area of research.

On the other hand, in computational methods, this is a parameter which tends to be the least defined. Often, the simulations work under the assumption of either a “good” solvent or a “bad” solvent. If a solvent is good or bad depends on the system studied and if the included molecules “like” the solvent or not. That usually depends on polarity and hydrophobicity of the molecules and solvent settings.

Resource Three Activities

Activities

1. What is the difference between model membrane and membrane model?
2. Define the terms in vitro and in vivo.
3. Which are the major parameters that need to be addressed and controlled if possible, under experimental conditions?
4. Compare primary cell culture with immortal cell lines
5. Explain the principle of coarse graining when using simulations
6. Discuss advantages and disadvantages of common biophysical model membrane types.

Resource Three Further Reading

Explore

- https://youtu.be/-_zYr6XG6Go – In vitro methods to study antibacterial and anticancer properties of nanomaterials
- https://en.wikipedia.org/wiki/HeLa - Background reading to Hela Cancer Cells

Common Biophysical Techniques

Microscopic techniques

Conventional microscopy can only provide limited information due to the fairly low resolution, and it is only partially suitable for bigger vesicles. However, there are some advanced optical and electron-based microscopic techniques which can be used to study monolayers, bilayers and liposomes.

Monolayers can be examined by the Brewster Angle Microscope, which is using both a microscope and reflection images of a polarised light source. Lipid bilayers can be studied with fluorescence microscopy, or atomic force microscopy, which gives a raster of the surface profile. Liposomes are commonly visualised with electron microscopy.

Independent research

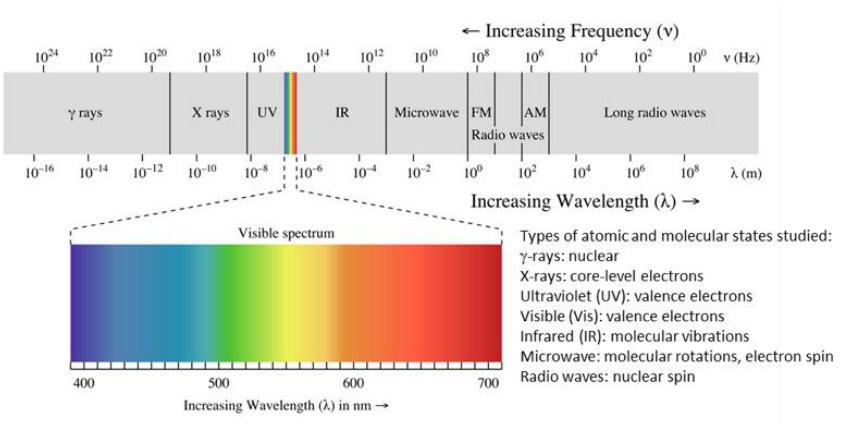
Spectroscopic techniques

Spectroscopy used the concepts of absorption and emission of light and other radiation by material when probed with electromagnetic radiation or light. It also can give information on the interaction between particles (i.e. electrons, protons, ions) and can therefore be used to study static states of membranes as well as dynamic processes, such as protein-membrane interactions.

Resource Three
Further Reading

Figure

The electromagnetic spectrum with boundaries between different regions and examples of spectroscopic techniques that are used to study different types of atomic and molecular states.



Vibrational spectroscopic methods such as Raman or Infrared are used to determine the chemical composition of both monolayer and bilayers, based on the detection of vibrational modes of the molecules. There are also a range of spectroscopic techniques based on scattering properties, such as X-ray and neutron scattering, but also light scattering that can be used on all model membranes mentioned.

Resource Four Overview

Topic	Can Lipid Monolayers Provide Biologically Relevant Information?
A-level Modules	Handling data, Graphs, Independent thinking Use and application of scientific methods and practices
Objectives	After completing this resource, you should be able to: ✓ Relate self-assembly of lipids to expected membrane formation. ✓ Propose model membranes for different cells or organisms and select suitable lipids ✓ Assess the results of basic monolayer experiments such as surface pressure measurement
Instructions	1. read the source 2. answer the questions 3. explore the further reading

Resource Four Data Source

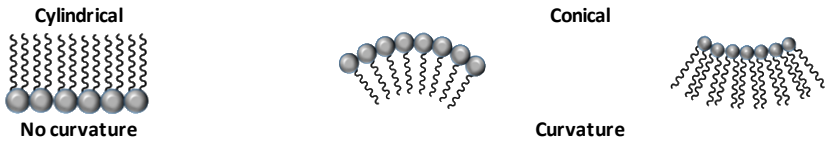
Section A

Lipid monolayers as a model membrane – Planning an experiment

This resource is focusing on using lipid monolayers as a model membrane to explore with biophysical techniques. Lipid monolayer experiments are usually done on the air-water interface to allow the lipids to self-assemble as a single planar leaflet. Therefore, the selection of lipids for making the monolayer should be made by considering their molecular geometry and favoured phase behaviour (also see source 1, lipid self-assembly) (figure 15).

Figure 15

Overview of common molecular geometry of lipids and resulting membrane curvature



Best suited for forming monolayers are cylindrical lipids such as sphingomyelin (SM), phosphatidylglycerol (PG) or phosphatidylcholine (PC). However, conical or inverted conical phospholipids such as phosphatidylethanolamine (PE) and phosphatidylserine (PS), or cholesterol can be used as well in a mixture with cylindrical lipids. The planning of an experiment should also take into account which type of membrane the monolayer is supposed to model and the approximate lipid composition of this membrane. Is the biological system of interest coming from the human body or an animal, the typically bacterial lipid PG should not be chosen. A membrane of a red blood cell for example has a different lipid composition from a neuron, and this type information can be found in the literature and should be considered when designing a model membrane. Similar is true for modelling bacterial membranes, when only lipids should be used that can be found in a bacterium, such as PG, PE or cardiolipin (CL).

Active reading

Resource Four Data Source

Looking at the table 9, it is obvious that a model membrane for the gram-negative E. Coli should be made of PE and PG only, whereas a model membrane for the gram-positive S. Aureus should only contain PG and CL as phospholipids. An important factor for complex model membranes is the lipid tail. Lipid headgroups are commercially available with different fatty acid tails, varying in length as well as saturation.

Table 9

Phospholipid composition of different bacterial membranes.

Phospholipid	Head net charge	E. coli	P. Aeruginosa	B. Subtilis	S. Aureus
PE	0	80 %	60 %	12 %	
PG	-1	20 %	22 %	70 %	58 %
CL	up to -2		11 %	4 %	42 %

Usually, experiments mimicking biological systems are performed under near-physiological conditions. That means the aqueous solvent, onto which the monolayer is going to assemble, is usually phosphate buffer at pH of 7 – 7.4 as this closest to the body conditions. Unfortunately, many techniques suitable for monolayer experiments do not allow temperature control and are therefore done at room temperature.

Active reading

Section B

Making monolayers

Before making a lipid monolayer, the powdered lipid needs to be dissolved first in an organic solvent, which in most cases is chloroform or a chloroform-methanol mixture. This lipid stock solution is normally made to a concentration ranging between 1 and 10 mg mL-1.

Resource Four
Data Source

To create a monolayer an aqueous-air interface is needed, which is given when using a Langmuir-Blodgett trough sample arrangement. The trough holds an aqueous subphase (phosphate buffer) and is equipped with two barriers that can be moved sideward to control the surface area (**figure 16**). Furthermore, the surface pressure (in mN m-1) of the solution is monitored with the help of the Wilhelmy plate method, which is a setup composed of a thin paper filter plate attached to a microbalance (= surface pressure sensor).

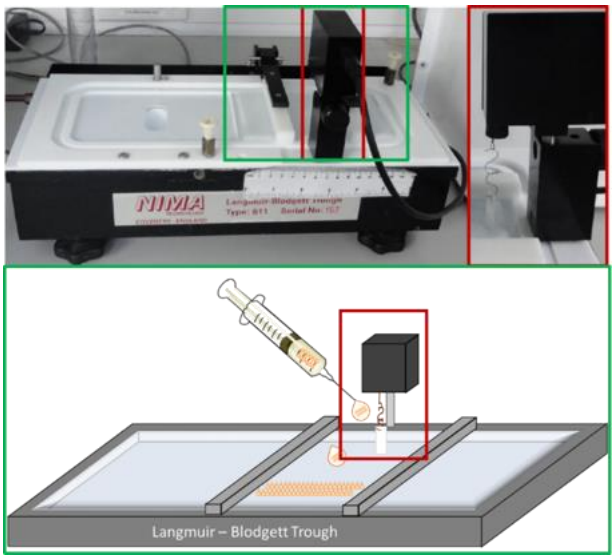


Figure 16

Langmuir-Blodgett trough with a surface pressure sensor using the Wilhelmy plate method. On the top a photo of an actual sample set up and a close-up of the surface pressure sensor. The area framed in green is shown below in the simplified illustration of the setup. The syringe is indicating the process of lipid monolayer making.

In reality, this method measures the force caused by surface tension (γ) acting on the Wilhelmy plate when partially immersed in the subphase. With the help of the plate dimension the force is then converted into the surface tension.

Surface pressure (p) is equal to the surface tension of a clean water (or buffer) interface (γ_0) subtracted by the surface tension measured (γ).

$$p = \gamma_0 - \gamma$$

Resource Four
Data Source

Once the subphase is ready, the surface pressure is normalised to Zero and the monolayer is made by carefully dropping microliters of the prepared lipid solution onto the buffer surface between the barriers. The lipid will spread itself out like an oil film on a water surface, but because of the fixed area within the barriers the molecules will start to self-assemble loosely into a monolayer with the hydrophobic tails facing the air.

After allowing time for the solvent (chloroform or chloroform – methanol mix) to evaporate, the surface area can be slowly reduced with the help of the barriers and the lipid assembly will become more ordered due to decrease molecular space and lipid packing. From the initially unordered (gaseous) state the monolayer undergoes changes to the more ordered liquid state and finally achieves the most ordered state and forms a solid monolayer (**figure 17**). This solid layer usually occurs when the lateral pressure similar to that of a cell membrane and this is the state the lipids are held in for further experiments.

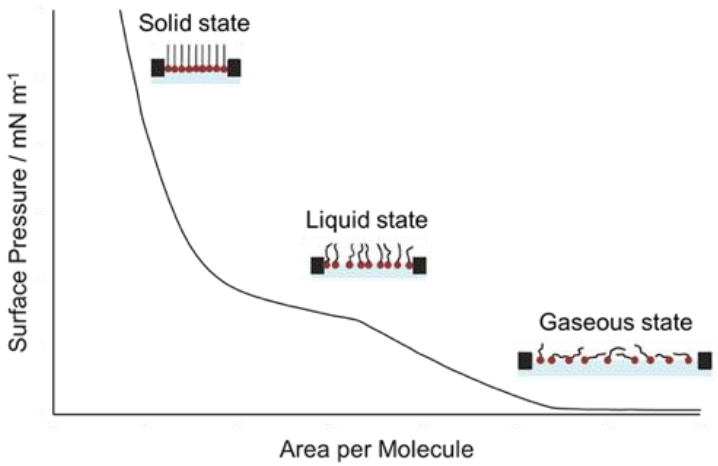


Figure 17

Compression isotherm of a cylindrical lipid. Phase changes in lipid ordering are clearly visible in the trace. The area per molecule is closely related to the surface area available to the lipids.

Naturally, different lipids and lipid mixes will undergo those phase transitions at different areas per molecule, depending on their own geometry, and not always all three states can be observed.

Resource Four
Data Source

Section C

Lipid Monolayer Experiments

Active reading

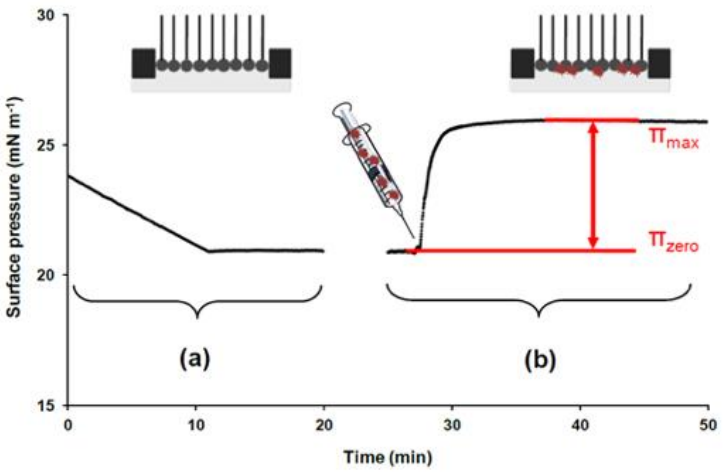
The monolayers can either be used to study lipid assembly on its own or as a model membrane to study interactions of molecules (i.e. proteins, drugs) with the lipid layer. This can be done by using the surface pressure measurement technique only or by coupling the trough with other instruments such as a Brewster Angle Microscope or an Infrared Spectroscope.

Example: Studying interactions of test molecules with lipid monolayers

Here, the monolayer is held at a constant area and the changes in surface pressure are monitored until the surface pressure stabilises. Once the surface pressure (and therefore the monolayer) remains stable (**figure 18 a**), a solution with the molecule of interest is introduced into the buffer subphase underneath the barriers without disturbing the lipid layer. If the molecule is interacting with the monolayer, say by binding to the headgroups or inserting between the lipids, a change in surface pressure can be seen (**figure 18 b**). Sometime those changes can be rapid or happening very slowly, depending on the type of interaction that causes the change. For this reason, this type of experiment is set up over a longer course of time in order not to miss any of the potential interaction.

Figure 18

Recording the surface pressure during the introduction of a test solution and changes happening over time. A) Relaxation of the lipid monolayer and pressure stabilisation. B) Introduction of a test molecule caused an interaction with the monolayer – pressure increase.



Resource Four
Data Source

But what can the surface pressure tell us? Whilst it is not a visual technique itself, we can draw a number of conclusions knowing the exact experimental input parameters. We know parameters such as structure and charge of the lipids but also a number of properties of the test molecules, i.e. concentration, chemical structure, charge, polarity, molecular weight.

The first question to answer is if there is a change in surface pressure at all. If the surface pressure remains stable at its initial value (**figure 19**), it can mean the molecule adsorbs at the monolayer but does not bind to it in a way that causes changes in the lipid layer packing and lateral pressure. It can also mean the molecule does not interact at all and is randomly distributed in the phosphate buffer subphase. Either way, in this case the technique cannot tell us anymore and a different technique or model membrane and needs to be trialed.

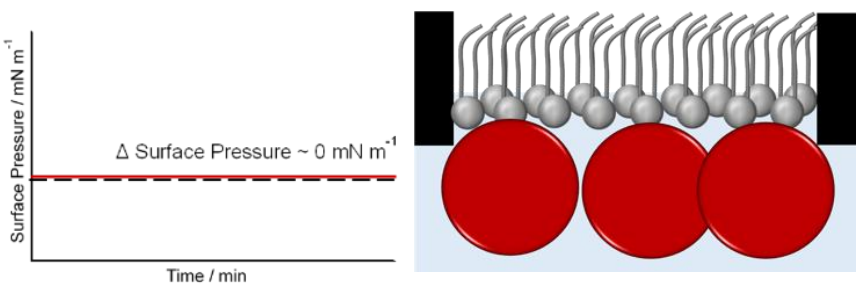


Figure 19

Final result: no changes in surface pressure. Molecules are not interacting with or only adsorbing onto the monolayer.

If there are changes in surface pressure visible, further evaluation of the results can be done. The net change in surface pressure can be calculated, which gives some indication about the strength of interaction.

Resource Four
Data Source

That can be useful for studying concentration dependence or for comparing the interaction at various conditions, i.e. under different pH. The computer-controlled setup is not only measuring the surface pressure but also the exact surface area enclosed by the barriers and computes the area per molecule by using parameters such as molecular weight and concentration of the lipids. With those quantitative results, conclusions on the amounts of molecules interacting with the lipids can be made.

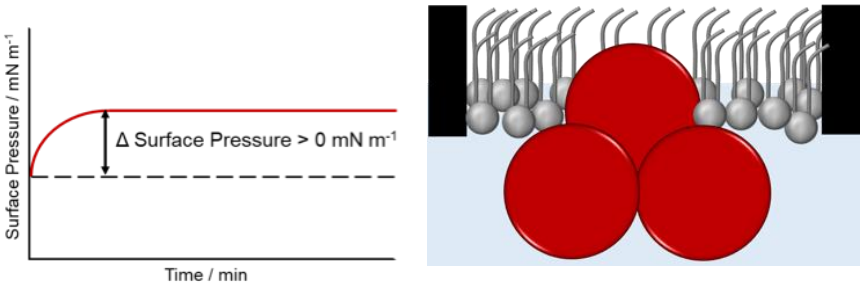
The observed changes can also be evaluated from a more qualitative point of view. Factors like the shape of the slope of the pressure increase, plateaus, multiple changes or even decrease in surface pressure can give clues towards the processes underlying the interaction of the molecule with the monolayer.

However, the simplest case is shown in **figure 20**, an increase in surface pressure followed by a plateau of constant surface pressure. The increase is caused by changing forces at the surface area caused due to penetration or insertion of molecules into the lipid monolayer. The plateau phase indicates the monolayer is back to an ordered state without any more changes. This means either all molecules have penetrated into the lipid layer and nothing else is happening anymore, or the lipids are so tightly packed now, that there is no more space for any more test molecules to insert into the layer.

Resource Four Data Source

Figure 20

Final result: increase in surface pressure. Penetration of the molecules into the monolayer cause increase in lipid packing and therefore decreases area per molecule.



There is another scenario of change that is not illustrated, which is the decrease in surface pressure down to the initial surface pressure of the phosphate buffer. This decrease means the monolayer is collapsing, which can be caused by a very active molecule (i.e. an antibiotic) penetrating rapidly and thereby disturbing the lipid monolayer so much that it cannot maintain its integrity. Alternatively, the initial lateral pressure is already very high due to dense lipid packing and small area per molecules, so that any small changes in the system (i.e. fluctuations in the barrier position, additional molecules) result in instability of the layer and collapsing of the membrane.

Whilst nowhere close to a real life, this monolayer model approach provides the opportunity to study aspects that could affect a biological system. No matter if the focus is on the sample environment, the behaviour of and interactions between the lipids, or the activity of and interaction with other test molecules; with lipid monolayers parameters can be easily adjusted and studied individually. Furthermore, it offers a platform to study factors that cannot easily be implemented in biological in vitro setups, such as pH or effect of organic solvents. It also can be used for screening of activity of test molecules in a particular system, and for prediction of their behaviour in more complex model membranes.

Resource Four Activities

Activities

1. Which lipid geometry is best suited to create monolayers?
2. In which state of lipid ordering is advisable to use for biophysical experiments.
3. Is surface pressure the same as surface tension? Explain your answer
4. Select lipids you would use to mimic a bacterial membrane.
5. Speculate on the potential interaction of test molecules with monolayers held at liquid state and at solid state of lipid order. Where would you see a stronger impact in the results of the surface pressure measurements and why?

Resource Four
Further Reading

Explore

- https://www.youtube.com/watch?v=sns1C1cK5EU
  Discovery of molecular monolayers
- https://www.youtube.com/watch?v=JgpphZE5XAk My own screen recording of an experiment, using surface pressure measurement combined with Brewster Angle Microscopy. In the high-speed recording of the screencast the different lipid order states during barrier compression can be seen. The model lipid used was a PG lipid, with fully saturated tails with 16 carbons.

Resource Five Overview

Topic	Lipid Bilayers – A step closer to "Real life"?
A-level Modules	Handling data, Independent thinking, Use and application of scientific methods and practices
Objectives	After completing this resource, you should be able to: ✓ Discuss the most common bilayer membranes ✓ Demonstrate a basic understanding of the neutron reflectometry technique for membrane research and its contribution to solve research questions
Instructions	1. read the source 2. answer the questions 3. explore the further reading 4. move on to the next resource

Resource Five Data Source

Section A

Lipid bilayers – Increasingly complex membranes

As biological membranes themselves are very complex, many different simplified but well-controlled systems have been developed, which contain the essential lipid bilayer structure and potentially other membrane components. The adaption of those lipid bilayers can result in different levels of complexity of the model membrane which then can be studied with a range of biophysical techniques. This resource will take a closer look at the most common model bilayers and provides insights to a technique called Neutron Reflectometry.

Liposomes

Slightly more complex, but easy-to-make bilayers are liposomes and vesicles ranging in the size of a few nanometres to microns. In general, liposomes are defined as spherical vesicles consisting of one or more lipid bilayers surrounding an aqueous core (**figure 21**).

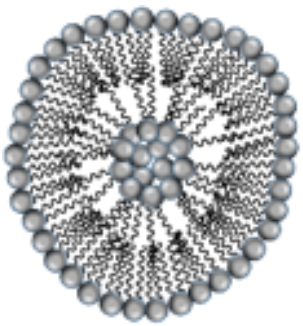


Figure 21

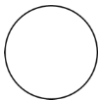
Schematic of a unilamellar vesicle.

Vesicles with more than one bilayer are called multilamellar vesicles (MLV), and vesicles with one bilayer only (unilamellar) are usually distinguished and referred to by size (small – SUV, large – LUV, giant – GUV) (see **table 10**). There is another variant of the unilamellar vesicles, which is made of multiple smaller unilamellar vesicle in the core of a giant one. This particular system can be used as a model for compartments within a cell.

Resource Five
Data Source

Table 10

Classification of Liposomes

Small unilamellar vesicles (SUV)	Large unilamellar vesicles (LUV)	Giant unilamellar vesicles (GUV)	Multivesicular vesicles (MVV)	Large multilamellar vesicles (MLV)
				
20 nm – 100 nm	100 nm – 400 nm	1 micron and larger	200 nm - ~ 3 microns	200 nm - ~ 3 microns

Liposomes are not only used to study membrane processes and interactions but are also increasingly used therapeutically in the medical and pharmaceutical field as carrier system for drugs, proteins/peptides and nucleic acids (gene therapy). Those liposomal drugs are developed and approved for wide range of applications, for example cancer therapy, as viral vaccines or medication for fungal diseases and pain. The application as carrier is because the hydrophilic compounds entrapped by a liposome cannot pass easily through the hydrophobic core of the lipid bilayer of a biomembrane on their own, and therefore use the liposomes and attached glycosylated membrane proteins as a vehicle to get to the desired cells. Once there, they content of the liposomes can be released for example by fusion of the liposomal lipid layer with the membrane of the target cell.

Supported Lipid Bilayers

These lipid bilayers can be planar bilayers sitting on a solid hydrophilic substrate (=support) either directly or on top of a connective layer called tether (**figure 22**). Because of this arrangement, only the upper leaflet of the bilayer is freely exposed to the surrounding solution, which has advantages and disadvantages. One of the major advantages is the stability of the bilayer provided by the solid support material (i.e. silicon, gold).

Resource Five
Data Source

Changes in the system such as high flow rates, vibrations or presence of holes will not destroy the entire bilayer and it can be kept for experiments lasting weeks or even months. Since the bilayer is on a flat hard surface, it can be characterised by a range of techniques that would not work on floating systems like monolayers. However, a major disadvantage is that inserting large trans-membrane proteins in the bilayer composition can lead to the reduction of their lateral mobility and function, and they also might adsorb onto the support. This problem can be overcome by using tethers to connect the lipid membrane with the solid support, and thereby making it behave more like a freely floating membrane and allow the membrane proteins to act more naturally.

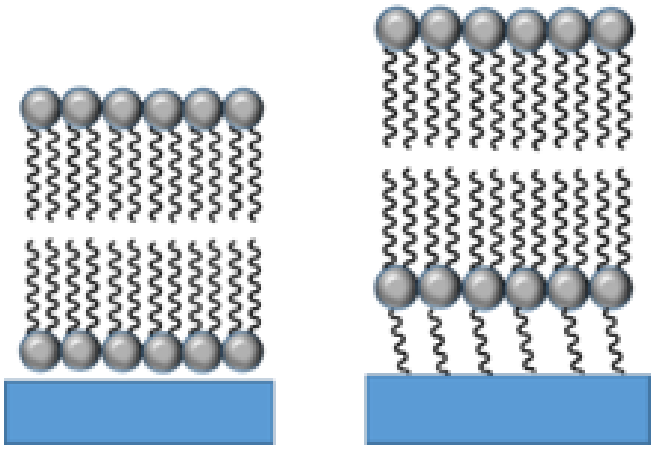


Figure 22

Schematic of a supported bilayer with the lipids sitting directly on the solid support (left) or being tethered by a linking molecule (right).

Section B

Neutron Reflectometry to study supported lipid bilayers

Active reading

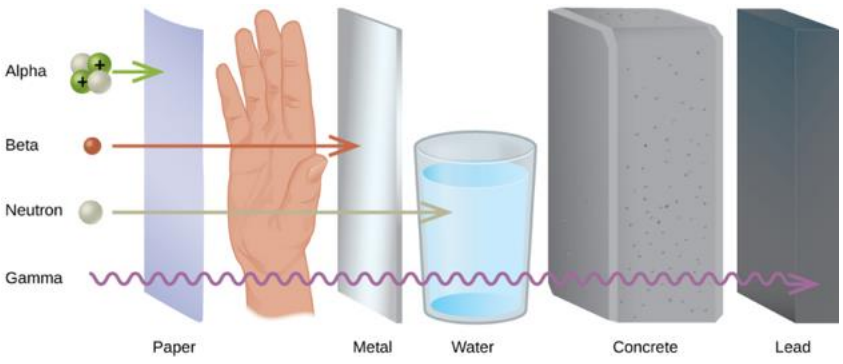
Lipid bilayers can be explored with a range of spectroscopic techniques and reflectometry is one of them. Reflectometry experiments can be done with light, X-rays or neutrons and here, we focus on neutron reflectometry which is generally used to explore the structure of thin films. The technique can provide valuable information over a wide of applications including chemical aggregation, polymer and surfactant adsorption, structure of thin film magnetic systems, biological membranes, and many more.

Resource Five
Data Source

What is a neutron?

A neutron is particle that can be found in atoms, together with protons and electrons. It is electrically neutral and forms together with the proton the atomic nucleus. Neutrons are comparatively heavy particles and have a mass of nearly 2000 times that of an electron. The number of neutrons in the atom are defining its isotope. For example, Hydrogen comes in three isotopes and they all have one proton and one electron. However, hydrogen has no neutron, deuterium has one, and tritium has two neutrons and therefore the isotopes have mass numbers of one, two, and three, respectively. Their nuclear symbols are therefore 1H, 2H, and 3H. Deuterium and tritium are extremely rare in nature.

Neutrons are stable when bound in the nucleus but have a lifetime of only ~ 1000 s as a free particle, which affects how far they can reach. Because neutrons are electrically neutral, they can penetrate more deeply into materials than electrically charged particles of comparable kinetic energy (figure 23). They reflect (scatter) from materials by interaction with the nucleus of an atom but not with the electrons.



By OpenStax - https://cnx.org/contents/havxkyvSZIPDownload:https://cnx.org/exports/85abf193-2bd2-4908-8563-90b8a7ac8df6@9.524.zip/chemistry-9.524.zip, CC BY 4.0, https://commons.wikimedia.org/w/index.php?curid=64301360

Figure 23

Differences penetration of alpha, beta, gamma and neutron radiation. Alpha particles are Helium nuclei consisting of two protons and two neutrons and are stopped by a sheet of paper. Beta particles are electrons and come to halt at an aluminium plate. Gamma radiation are electromagnetic waves (energetic photons) that are dampened when they penetrate material. Gamma rays are similar to X-rays but have higher frequencies and can be stopped and absorbed by dense material such as lead. The free neutron particles can be blocked by light elements such as hydrogen (or water), which slow them down and /or capture them.

Resource Five
Data Source

Neutron cross-section

How much a neutron interacts with the nucleus is called cross-section (scattering power). Neutron cross-section is the effective area presented by a nucleus to an incoming neutron and is therefore a measure of probability of those two particles colliding. The larger the nuclear area, the higher the probability of collision. The nuclear dependence of the scattering means isotopes of the same element can have significantly different cross-sections. This makes hydrogen and deuterium (heavy water) popular isotopes to label different molecules and thereby increase the contrast and visibility in neutron experiments. The neutron cross-section is also used to calculate a parameter called scattering length density, which is also different for each element.

What is neutron reflectometry?

Neutron reflectometry uses the reflection of a neutron beam at flat surfaces and interfaces; where the angle of the incident beam is equal to the reflected beam. The intensity of the reflected beam results in a reflectivity profile which can converted to a scattering length density profile that can give detailed information about the structure of the surface, including the thickness, density, and roughness of any thin films layered on the solid substrate (see **figure 24**).

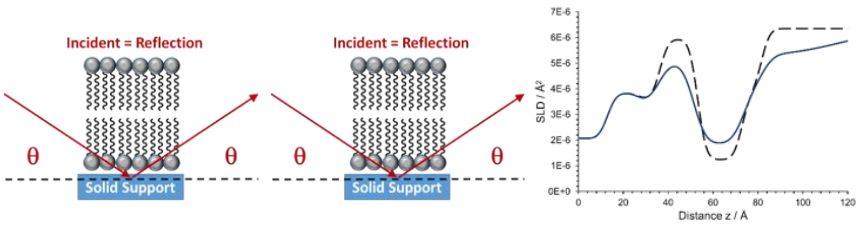


Figure 24

Principle of neutron reflectometry shown at the example of a lipid bilayer. The angle of neutron incident beam is equal to the angle of reflection. The amount of neutron absorption of the membrane will affect the number of neutrons in the reflected beam and the resulting intensity in reflectivity profile. An example reflectivity profile is shown in the middle, where the black profile presents a lipid bilayer, and blue profile the same lipid bilayer after interacting with a test molecule. The reflectivity data are used calculate scattering length densities (SLD) which can be plotted over the distance (thickness) of the lipid bilayer and provided further information on changes to the layer..

Resource Five
Data Source

The reflection is described as a momentum transfer vector which is defined as change in momentum of a neutron after reflecting from a material. The momentum transfer is dependent on the neutron wavelength and the angle of incident ( ). The wavelength of the neutrons used for reflectivity are typically on the order of 0.2 to 1 nm (2 to 10 Å). As neutron scattering is sensitive to contrast coming from different nuclei, it allows the technique to differentiate between various isotopes of an element, which vary in the number of existing neutrons in the nucleus and therefore in the area the nucleus offers for interaction (=> neutron cross section).

Using contrasts to identify structures – principle of contrast matching

When planning a neutron experiment it is important to think about the potential limitations in the system, for example, are there any elements that have similar cross sections and therefore cannot be distinguished easily by neutrons? What is the surrounding solvent made of and how would it scatter?

If we use lipids as an example, they have hydrocarbon tails and therefore a fairly high number of hydrogens. If we want to look at the lipids in phosphate buffer, which also contains many hydrogens, we will likely not see much difference in the reflectivity profile of both and can only identify the lipid heads as they have a different reflectivity profile due to their different elemental composition.

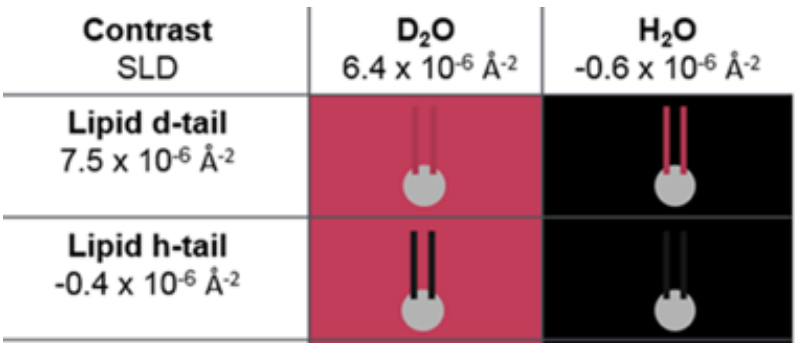


Figure 25

. Illustration of the principle of contrast matching. SLD stands for scattering length density and is a parameter calculated from the neutron cross section and reflectivity profile. The bigger the difference of that parameter between two components of the system, the higher the contrast and the better visibility to the neutrons. D2O is heavy water made with deuterium (H2 Isotope of hydrogen). Lipid d-tail means that some of the hydrogens of the hydrocarbon tail are replaced (labelled) with deuterium; whilst lipid h-tail mains the lipids have a normal hydrocarbon tail.

Resource Five
Data Source

Therefore, two different type of solutions are used – normal water and heavy water (D2O) which have a big scattering difference, and two variants of the same lipid – one with a normal hydrocarbon tail, and one where the hydrogens are replaced with deuterium. Figure 25 is illustrating that different parts of the lipid can be made "visible" to the detector by using different contrast combinations. This principle of contrast matching can be applied to other systems too.

Making lipid bilayers

A neutron experiment should be planned very carefully to make the most of the technique. Once the decision is made on which lipid composition is to be studied under which experimental conditions (pH, temperature, solvent), the components visible and invisible to neutrons needs to be identified. Is there enough scattering contrast between the molecules and their surrounding system? If not, how can this contrast be increased, i.e. by using deuteration of the solvent and/ or the system? If the molecules have parts that scatter different from the rest of the molecule, those sections might need labelling too to make them more visible.

The next question is how to make the bilayers that need to be hold in a closed sample cell for neutron reflectometry? For this particular type of experiment, supported bilayers are needed which can be made in multiple ways. One way, which is well-controllable, is to use the monolayer technique (=> resource 5) and transfer the monolayer onto a solid support, and then pass this supported monolayer on top of a new monolayer to assemble a lipid bilayer (figure 26). This method can be used with lipids with hydrogen in the tails as well as with lipids with deuterated tails to increase the contrast.

Resource Five
Data Source

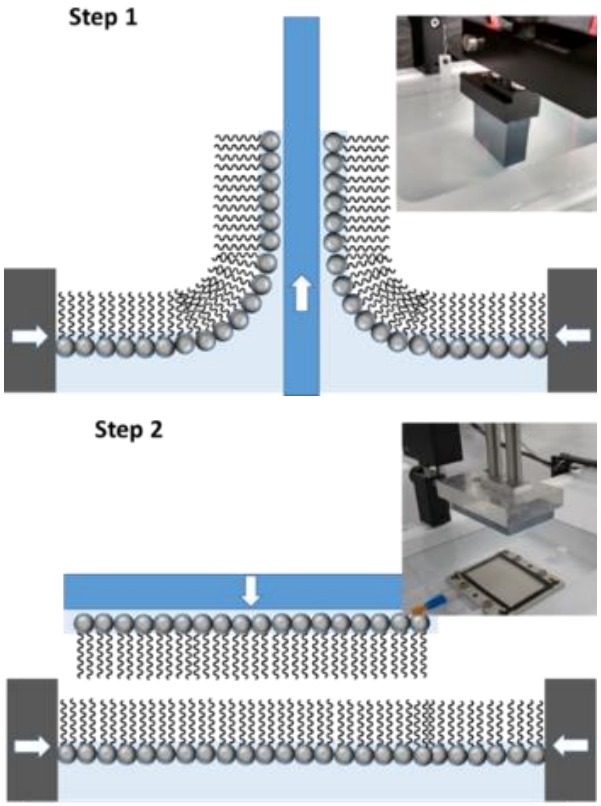


Figure 26

Making supporting bilayers with the help of the monolayer technique. In step 1, the monolayer is held at a constant pressure with the help of the barriers and a hydrophilic support substrate is pulled from the aqueous subphase upwards to transfer the first monolayer (see photo). Then, at step 2, the support substrate with the first monolayer on it is pushed horizontally onto the second monolayer to collect it and assemble it on top of the first one (see photo), and thus the supported bilayer is ready and can be enclosed in the sample cell which lower part is visible in the bottom left of the photo.

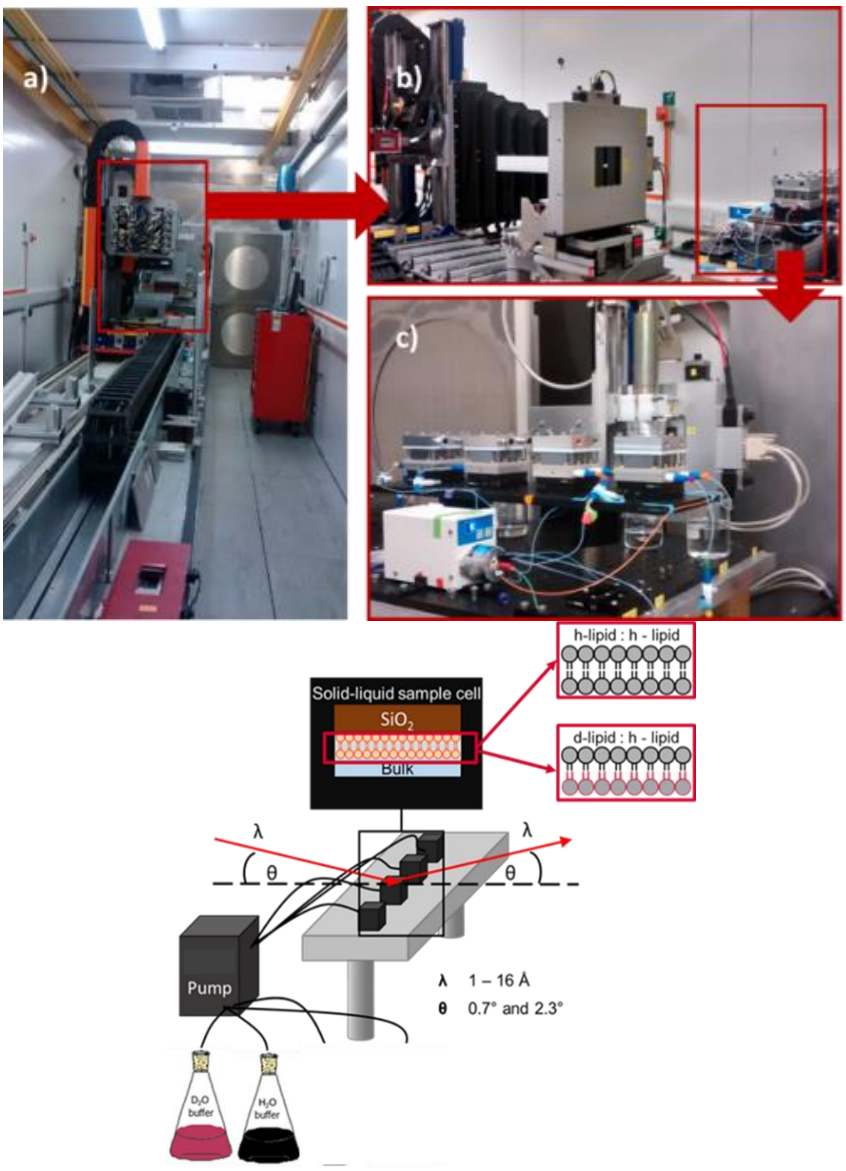
Resource Five
Data Source

Figure 27

Pictures taken at a neutron reflectometer at the Neutron Spallation Source at Harwell. Picture a) looks into the experimental chamber. The actual experiment is set up at the end of the chamber (red box), and the neutron beam comes from the source (behind the back wall) guided through slits to adjust the size of the beam hitting the sample cells. In panel b) the detector can be seen in the right and middle of the photo (grey square attached to the black plastic construct). Next to it on the right, the sample cells can be seen and are enlarged again in panel c). Also visible in this photo is the pump connected to the sample cells as well as to the buffer solutions. A simplified clearer illustration of this setup is shown next to it. Two different lipid bilayers are needed, one which only contains lipids with hydrogen in the lipid tails (h-lipid), and one which has one leaflet made of lipids with deuterium replacing the hydrogen of the lipid tails (d-lipid). The supported bilayers are enclosed by the sample cells connected to a pump, which can pump D2O or H2O buffer into the sample cell. The neutron beam is targeted to the sample cell at different fixed angles (0.7 and 2.3° in this case) and over a certain wavelength range (1 – 16 Å) and the reflected beam is collected to create a reflectivity profile.

Experimental setup

When the lipid bilayers are created, they are stored in solid-liquid sample cells, which are closed environments and contain an area for the buffer solution and some valves. The neutron reflectometer is an automated system, which means multiple samples cells (up to 4) can be installed on the sample table and tested one after the other with the same instrumental conditions using a robotic approach, without needing to change anything manually.



Resource Five Data Source

The sample cells can be connected to pumping system to flush different buffers (based on water and heavy water) through and therefore change the contrast conditions around the supported lipid bilayer (**figure 27**).

Once everything is set up as needed, the experiment can be started and controlled by a computer in a different room. Due to potential radiation damage, the experiments always take place in a locked room where no one is allowed in once the neutron beam has been switched on.

What can we learn from a reflectivity profile?

As neutron reflectometry is a spectroscopic technique, we do not get any visual images but a profile of the intensity of the neutron beam reflected from the surface which is called reflectivity. The measured reflectivity values are converted into scattering length densities (SLD) which are plotted against a distance and can be used to obtain information regarding the thickness, packing density, hydration and roughness of the lipid bilayer.

The easiest way to approach the graphs, is to imagine a lipid bilayer and divide it into subsections: solid substrate with a hydration layer, lipid headgroups of the inner leaflet, lipid tails of the inner leaflet, lipid tails of the outer leaflet, lipid headgroups of the outer leaflet, and a bulk area with buffer or test molecules.

Resource Five
Data Source

Due to its chemical composition each subsection will absorb and reflect neutrons differently and therefore create changes in the measured SLD. Knowing the theoretical values we can then match areas of the profile to our bilayer subsections (**figure 28 left**). If we want to study interactions of test molecules with the lipid bilayer, we measure the lipid SLD profile before and after exposure to the test compound (**figure 28 middle, right**) and from changes in the profiles we can conclude what happened on a nanoscale basis. Using the principle of contrast matching we can extract even more detail, for example which subsections of the lipid bilayer are involved in the interaction, and other information such as lipid packing and lipid exchange across the membrane.

Linking these observations of membrane penetration and adsorption with results from other techniques such as surface pressure measurements, can provide comprehensive insights to membrane structures and processes happening there, and therefore it is a very powerful technique for membrane studies.

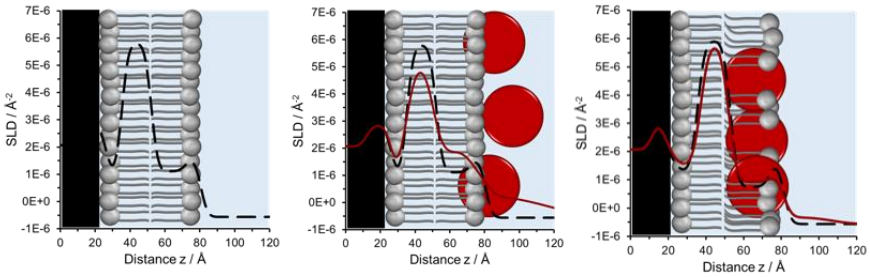


Figure 28

Neutron scattering density (SLD) profiles of a pure lipid bilayer on its own (left), and after interaction of a test molecule with the lipid bilayer (in red, graphic in the middle and on the right). It is shown only one of four possible contrasts. The graphic in the middle is showing molecules adsorbing at the headgroups and building up an additional layer which can be seen in the changes of SLD between 80 – 120 Å and showing an increased thickness of the system. It also inserts into the outer lipid leaflet affecting the SLD of the outer lipid headgroup and tails. The changes seen in the SLD of the tail areas (which is mainly affected by hydrogen scattering) are due to the disturbed lipid bilayer and therefore more aqueous buffer into that area. The graphic on the right shows the changes in SLD caused by molecules penetrating into the lipid bilayer. The differences of the SLD traces are more subtle in this case, the overall thickness of the bilayer is not changing much. There are some changes in the SLD of the outer headgroup (70 – 80 Å) layer which are caused by the inserted molecules in that subsection. As the visible changes are mainly in the outer membrane leaflet it can be concluded that this particular molecule only interacts with that side of the membrane, but the interaction is not strong enough to destroy the lipid bilayer or affect the inner leaflet.

Resource Five Activities

Activities

1. List the classes of liposome.
2. What is a neutron?
3. List the isotopes of Hydrogen and explain the difference between them
4. Compare a small unilamellar vesicle with a multilamellar vesicle
5. Applying the principle of contrast matching, which parts of a lipid are likely to be visible when we use normal lipids with hydrocarbon chains and a phosphate buffer made with D2O?

Resource Five
Further Reading

Explore

- Examples of membrane and lipid research using Neutron Reflectometry.
  https://www.isis.stfc.ac.uk/Pages/Mastering-Membrane-Mimicking-to-Outsmart-Bacteria.aspx
  https://www.isis.stfc.ac.uk/Pages/SH19_Rethinking-lipid-transfer-in-bacteria.aspx

- Video of using neutron scattering to image the coffee making processing in an espresso cooker.
  https://www.youtube.com/watch?v=VESMU7JfVHU

Resource Six Overview

Topic	Using lipid membrane models for researching new medicines
GCSE Modules	Handling data, Graphs, Independent thinking
Objectives	After completing this resource, you should be able to: ✓ Illustrate the importance of membrane-related research for the study of new medicines ✓ Evaluate the experimental results in a lipid-focussed context.
Instructions	1. read the source 2. answer the questions 3. explore the further reading 4. move on to the next resource

Resource Six
Data Source

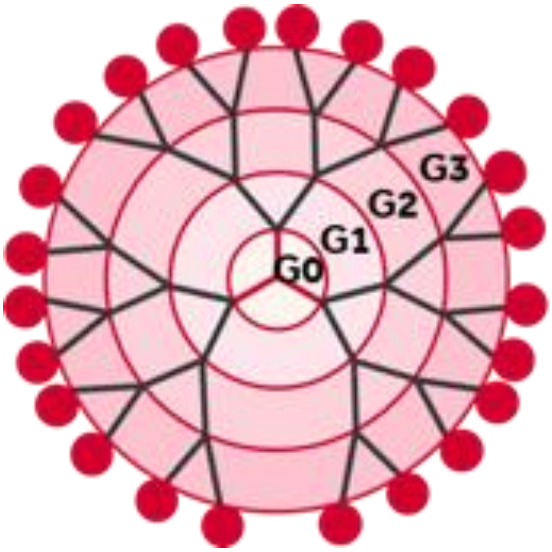
Section A

Branched polymers as drug-delivery systems

In this resource, we will focus a bit more on my own research and how the topics addressed in the other resourced link into it. I work with very regularly branched polymers (=dendrimers), which due their chemical structure can have various biomedical applications. The general architecture of a dendrimer is illustrated in **figure 29**.

Figure 29

General structure of a regularly branched polymer, which is made of units repeating themselves at each branching point. The red dots on the outside are the different surface groups. Every time the polymer branches again it creates another layer, called generation. In this illustration G0, G1, G2, and G3 are the names of the generation.



Initially, they were developed as a potential drug-delivery system or nano-sized carrier to help delivery medicines that do not pass membranes easily, have lots of strong side effects or are excreted from the body too quickly to get to the areas where they are supposed to action (i.e. cancer cells). They were designed with a range of different surface groups which can bind to medicines, proteins and peptides or nucleic acids (**figure 30 left**). The branched structure of the molecules also allows substances to be trapped in its core (**figure 30 middle**). Over time, research showed that the polymers have some biological activity themselves, without binding to any therapeutic substances (**figure 30 right**).

Active reading

Resource Six
Data Source

Figure 30

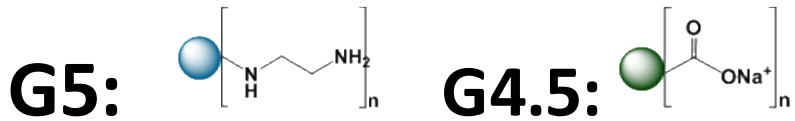
Concept of a drug carrier. The polymer acts as taxi service for therapeutic compounds, either by connecting them to surface groups or by enclosing them within the branches of the polymer core. It can also be therapeutically active on its own or combine its own activity with that of the medicines it carries.

As these polymeric carriers have a large potential for a variety of biomedical applications, it is important to understand how they are working in the body. This is not only essential to make the useful for a wide range of medicines, but also to ensure that they are safe for patients and do not cause any unwanted side effects or even worse damage.

My research project mainly uses biophysical model membranes and in vitro cultures to study the biological effect and activity of two branched polymers with different surface groups. The polymers have 5 layers and take on a spherical shape and are therefore now referred to as G5 (amine surface groups, NH3) and G4.5 (carboxyl surface groups, COOH) (figure 31).

Figure 31

Simplistic illustration of two branched polymers with amine surface groups (G5) and carboxyl surface groups (G4.5)



Resource Six
Data Source

Section B

Interactions with biophysical model membranes

Active reading

First, I was screening the binding preference to different headgroups of cylindrical lipids using lipid monolayers and surface pressure measurements. It turned out that both of the polymeric carriers did not show much interaction with eukaryotic lipids, but with bacterial lipids. The bacterial lipid I was using had a phosphatidylglycerol headgroup and two palmatic acdids (16:0) making up its tail (see structure in figure 32).

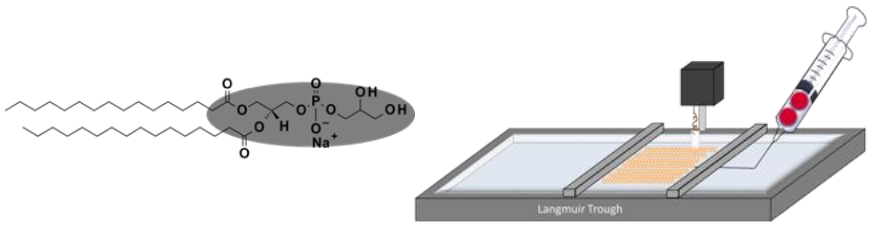


Figure 32

Phosphatidylglycerol lipid with palmitic acid tails and the illustration of the surface pressure measurement technique.

The polymers are provided in methanol, and for some techniques the methanol is removed before testing and for other techniques a residual of methanol remains when diluting the sample to the desired concentration. To understand if this methanol residual is a factor that affects the lipid interaction of the polymer molecules, I compared the changes in surface pressure with residual methanol present and absent in the system.

The results of the monolayer experiments (figure 33) showed the residual methanol did not influence so much the interaction of G5 (in blue). The interaction profile looks very similar, and the change in surface pressure is slightly higher. However, when looking at the interaction of G4.5 (in green) some interesting differences can be seen. For one, without the methanol present, there is no interaction with the PG lipid seen.

Resource Six
Data Source

Furthermore, not only does the presence of methanol seems to support the lipid interaction as the surface pressure increase indicates, the type of interaction is likely to be different to that of G5 too. It is a much slower interaction with the membrane and an initial slight decrease in surface pressure can mean that the stability and lipid arrangement of the monolayer was disturbed which then enabled the polymer to take up space in the membrane.

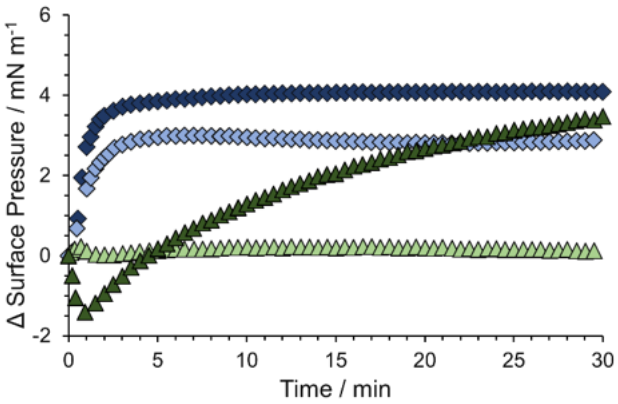


Figure 33

Surface pressure profiles of polymeric carriers interacting with PG monolayers on phosphate buffer at pH 7. The blue traces belong to G5 which has a positive surface charge at pH 7, and the green traces belong to G4.5 which has a negative surface charge at pH 7. The darker interactions profiles have ~ 1 % methanol residuals in the phosphate buffer, whereas methanol was completely removed from the phosphate buffer in the case of the brighter curves.

Next, we thought about the potential effect of the pH onto the polymer-lipid interaction. Here, we got some unexpected results. Thinking of the surface charge only, one would expect strong electrostatic interactions of G5 with the anionic lipid heads at pH 4, when the amines are all charged. In fact, we observed a very high and rapid change in surface pressure at pH 4 and a comparatively low change at pH 10.

However, it was a different story for G4.5, where we for one did not expect strong interactions due to the carboxyl surface groups but also, we expected a different pH trend. To our surprise, the G4.5 polymer with the supposedly uncharged headgroup at pH 4 did not only interact more than at pH 7, it also reached similar surface pressure changes as the positively charged G5.

Resource Six
Data Source

The result at pH 10 was also against our prediction. Despite the negatively charged carboxyl groups and expected electrostatic repulsion interactions, there were hardly any changes in surface pressure. After a small pressure drop in the beginning, the pressure increased very slowly again over time.

Both of the polymer carriers are exactly the same except for the surface groups, so what can those results mean?

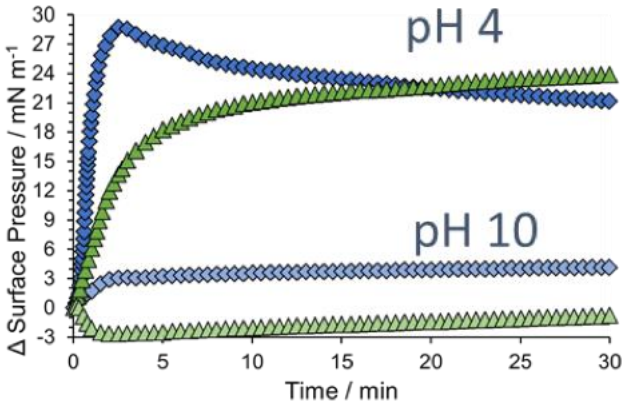


Figure 34

Surface pressure profiles of polymeric carriers interacting with negatively charged PG monolayers on phosphate buffer at pH 4 (top traces) and pH 10 (bottom traces). The blue traces belong to G5 (NH3), the green traces belong to G4.5 (COOH). The surface groups of G5 should be all ionised and charged at pH 4 and not charged at pH 10. In comparison, the surface groups of G4.5 should be all ionised at pH 10 and not charged at pH4.

We started investigating further, doing a range of pH-dependent biophysical studies of the polymer only in phosphate buffer as well as some molecular dynamics simulations. We also probed the effect of pH on more complex bilayer systems with neutron reflectometry. Those further studies confirmed the "strange" behavior of the G4.5 molecule, but also the preference in interaction with negatively charged headgroups. We believe that the membrane interactions and biological activity are not mainly driven by the reactivity of the headgroups as widely assumed. The chemistry of the core seems to play a bigger role than anticipated and the molecules have a flexibility within their branches to stretch and bend as a reaction to their chemical environment.

Resource Six
Data Source

Following the screening on monolayers and the found interaction preference to the bacterial lipid headgroup phosphatidylglycerol, I moved on to in vitro screening of different bacterial strains. I was studying which effect the pure polymers might have on the growth of bacterial cultures, looking at a range of typical gram-negative and gram-positive species that often cause infections.

When studying the growth of bacteria, the optical density is a common parameter used to describe the effect of active substances and is coming from the absorbance of light of the suspension culture. The more bacteria are in the culture, the cloudier the suspension is and the higher the absorbance intensity (optical density) measured. However, with this technique we cannot distinguish between life or dead cells, which makes the interpretation of results more difficult and often requires additional techniques.

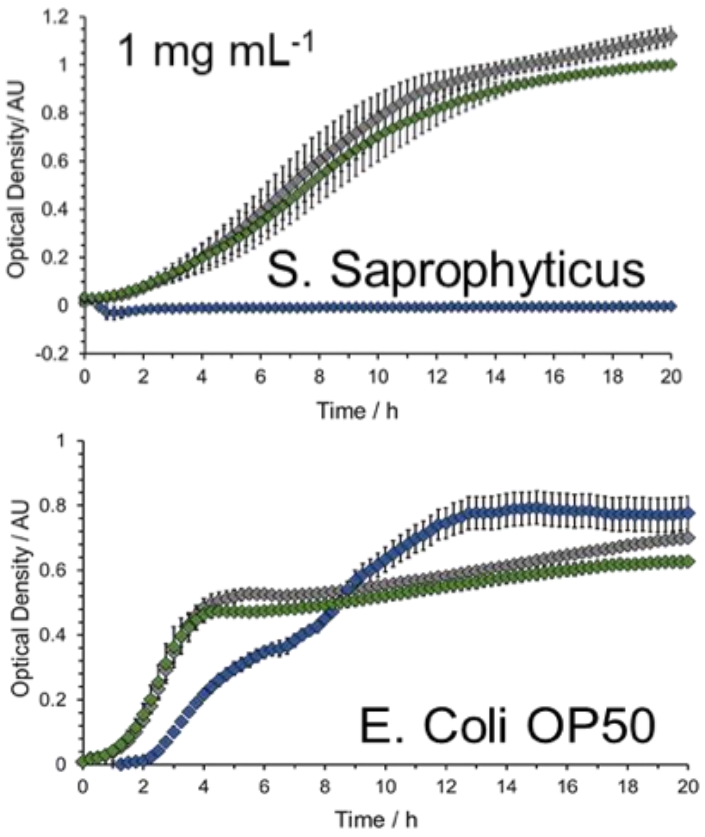
Active reading

I found with the optical density screening at physiological pH 7 and 37°C incubation that a lot of the bacterial strains showed activity of the G5 polymer by either a decreased optical density or a changed optical density-time profile compared to the control. The G4.5 polymer showed some activity, but not as strong, and for less bacteria than the G5 polymer. And overall for both polymers, the effect on gram-positive bacteria was stronger than on gram-negative bacteria. An example of the different effect is shown in **figure 35**.

Resource Six Data Source

Figure 35

Optical density screening on gram-positive Staphylococcus Saprophyticus and gram-negative Escherichia Coli. The grey trace indicates the untreated control culture, in blue polymer G5 and in green polymer G4.5. In the gram-negative S. Saprophyticus, G4.5 only has little effect, but the G5 keeps the optical density around 0 which means there is no growth of bacteria over 20 h. In the gram-negative E. Coli, the G4.5 does not have much effect but the onset of growth of the culture is delayed by G5, and it also shows a different profile compared to the control hinting towards adaptation processes happening within the culture.



Whilst the project is not finished at the moment, there are already some conclusions that can be drawn looking at the biophysical and biological membrane results. Thinking of the membrane structure of gram-negative and gram-positive bacteria, as well as some known lipid compositions, we can explain some of the strong interaction of the G5 polymer.

Resource Six Data Source

The phosphatidylglycerol lipid it was binding to in the biophysical studies, is one of the major membrane phospholipids of bacteria, and it is found in much higher portion in gram-positive bacteria compared to gram-negative bacteria (i.e. S. Aureus has 58 % PG and 42 % CL; E. Coli has 80 % PE and only 20 % PG). The higher presence of negatively charged PG (or CL) could offer a bigger interface for electrostatic interactions with the positively charged G5 polymer at pH 7. Furthermore, gram-negative bacteria have a second membrane as protective layer, that the polymers need to cross to cause damage to the cell and the lipid composition of the outer membrane might not offer many binding options.

Resource Six Activities

Activities

1. What is the difference between G5 and G4.5 polymer?
2. Explain the principle of a drug carrier system.
3. Can we know from the optical density how many bacteria are killed by the polymers? Justify your answer.
4. Why did I decide to screen the activity of the polymers on bacterial cultures?
5. I did a couple of biophysical experiments to study the interaction of the polymers with lipid model membranes. Can you identify which factors I studied, that may play a role in the membrane interaction?

Resource Six
Further Reading

Explore

- https://en.wikipedia.org/wiki/Dendrimer - more in depth information about a specific type of branched polymers

Final Reflection

Deep Dive

Final task: Imagine you are given a number of newly synthesised substances with unknown properties and unknown activity and asked to examine their biological activity.

Using your knowledge of lipids and membrane models, suggest a set of experiments you would do to test the activity. Include in your suggestion factors such as temperature, pH, type of lipids, type of membrane model etc.

Be as specific and detailed as possible using the help of the resources.

Explain the reasoning behind your chosen elements of the experiment.

You can either focus on activity towards the human body, towards bacteria or both.

Part 3 – Study Skills, Tips & Guidance

This section includes helpful tips to help you complete this pack, as well as improve your study skills for any courses you take next year.

It also includes a few fantastic easy-to-use resources to know what to do next if you are hoping to go to university in the next few years, like UCAS advice and web links to more academic opportunities.

In this section:

University Study Skills:

- ✓ Cornell Notes
- ✓ Key Instruction Words
- ✓ Academic Writing
- ✓ Referencing
- ✓ Evaluating Your Sources

University Guidance:

- ✓ What next?

Subject Guidance:

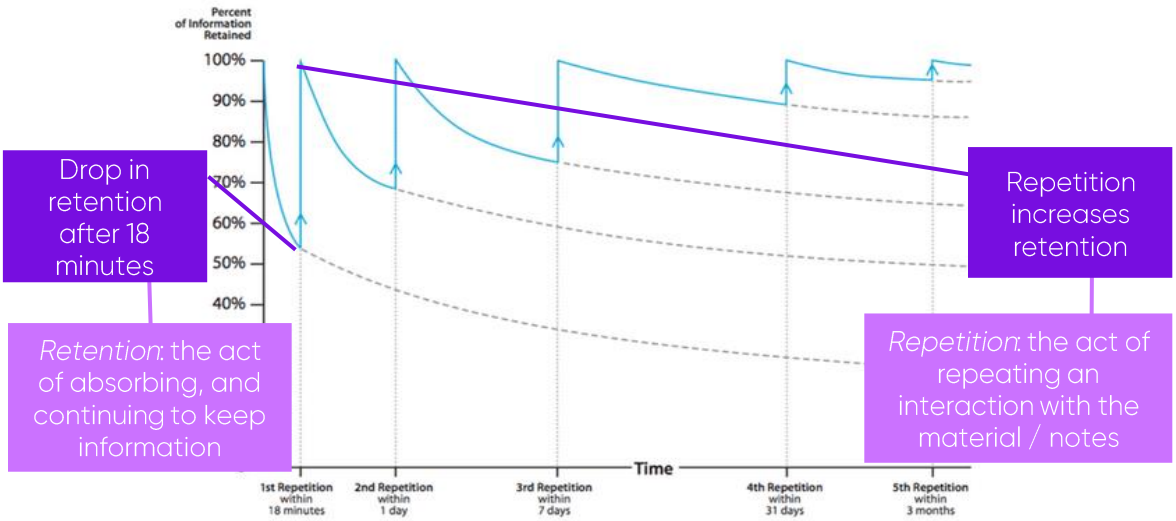
- ✓ More on studying your subject

University Study Skills
Cornell Notes

Why is good note taking important?

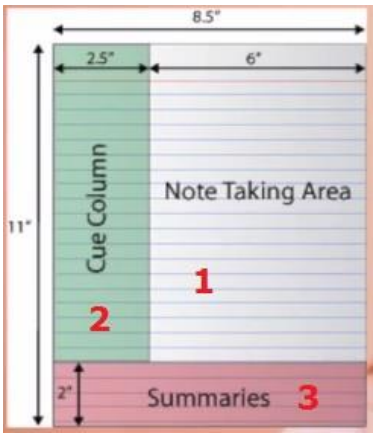
If it feels like you forget new information almost as quickly as you hear it, even if you write it down, that's because we tend to lose almost 40% of new information within the first 24 hours of first reading or hearing it.

If we take notes effectively, however, we can retain and retrieve almost 100% of the information we receive. Consider this graph on the rate of forgetting with study/repetition:



Learning a new system

The Cornell Note System was developed in the 1950s at the University of Cornell in the USA. The system includes interacting with your notes and is suitable for all subjects. There are three steps to the Cornell Note System.



Step 1: Note-Taking

1. Create Format: Notes are set up in the Cornell Way. This means creating 3 boxes like the ones on the left. You should put your name, date, and topic at the top of the page.

2. Write and Organise: You then take your notes in the 'note taking' area on the right side of the page. You should organise these notes by keeping a line or a space between 'chunks' /main ideas of information. You can also use bullet points for lists of information to help organise your notes.

University Study Skills
Cornell Notes

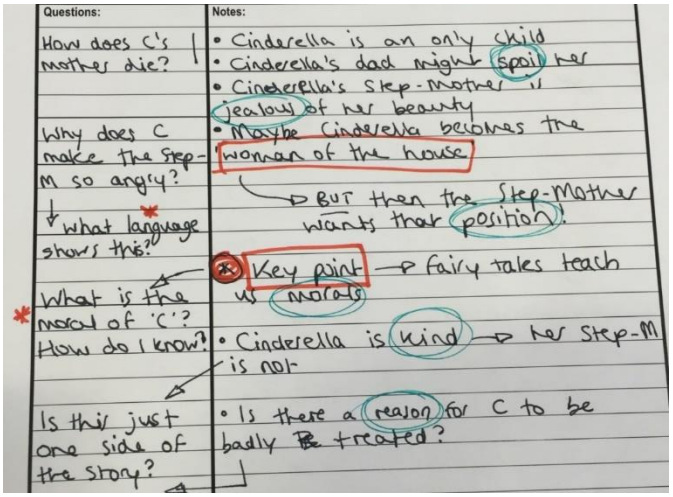
Step 2 Note-Making

1. Revise and Edit Notes: Go back to box 1, the note taking area and spend some time revising and editing. You can do this by: highlighting 'chunks' of information with a number or a colour; circling all key words in a different colour; highlighting main ideas; adding new information in another colour

2. Note Key Idea: Go to box 2 on the left hand side of the page and develop some questions about the main ideas in your notes. The questions should be 'high level'. This means they should encourage you to think deeper about the ideas. Example 'high level' questions would be:

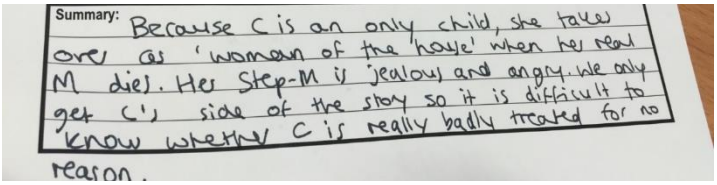
- Which is most important / significant reason for...
- To what extent...
- How does the (data / text / ideas) support the viewpoint?
- How do we know that...

Here is an example of step 1 and step 2 for notes on the story of Cinderella:



Step 3 Note-Interacting

1. Summary: Go to box 3 at the bottom of the page and summarise the main ideas in box 1 and answer the essential questions in box 2.



Give the Cornell Note Taking System a try and see if it works for you!

University Study Skills
Key Instruction Words

These words will often be used when university tutors set you essay questions - it is a good idea to carefully read instruction words before attempting to answer the question.

Analyse – When you analyse something you consider it carefully and in detail in order to understand and explain it. To analyse, identify the main parts or ideas of a subject and examine or interpret the connections between them.

Comment on – When you comment on a subject or the ideas in a subject, you say something that gives your opinion about it or an explanation for it.

Compare – To compare things means to point out the differences or similarities between them. A comparison essay would involve examining qualities/characteristics of a subject and emphasising the similarities and differences.

Contrast – When you contrast two subjects you show how they differ when compared with each other. A contrast essay should emphasise striking differences between two elements.

Compare and contrast – To write a compare and contrast essay you would examine the similarities and differences of two subjects.

Criticise – When you criticise you make judgments about a subject after thinking about it carefully and deeply. Express your judgement with respect to the correctness or merit of the factors under consideration. Give the results of your own analysis and discuss the limitations and contributions of the factors in question. Support your judgement with evidence.

Define – When you define something you show, describe, or state clearly what it is and what it is like, you can also say what its limits are. Do not include details but do include what distinguishes it from the other related things, sometimes by giving examples.

Describe – To describe in an essay requires you to give a detailed account of characteristics, properties or qualities of a subject.

Discuss – To discuss in an essay consider your subject from different points of view. Examine, analyse and present considerations for and against the problem or statement.

University Study Skills
Key Instruction Words

Con't

Evaluate – When you evaluate in an essay, decide on your subject's significance, value, or quality after carefully studying its good and bad features. Use authoritative (e.g. from established authors or theorists in the field) and, to some extent, personal appraisal of both contributions and limitations of the subject. Similar to **assess**.

Illustrate – If asked to illustrate in an essay, explain the points that you are making clearly by using examples, diagrams, statistics etc.

Interpret – In an essay that requires you to interpret, you should translate, solve, give examples, or comment upon the subject and evaluate it in terms of your judgement or reaction. Basically, give an explanation of what your subject means. Similar to **explain**.

Justify – When asked to justify a statement in an essay you should provide the reasons and grounds for the conclusions you draw from the statement. Present your evidence in a form that will convince your reader.

Outline – Outlining requires that you explain ideas, plans, or theories in a general way, without giving all the details. Organise and systematically describe the main points or general principles. Use essential supplementary material, but omit minor details.

Prove – When proving a statement, experiment or theory in an essay, you must confirm or verify it. You are expected to evaluate the material and present experimental evidence and/or logical argument.

Relate – To relate two things, you should state or claim the connection or link between them. Show the relationship by emphasising these connections and associations.

Review – When you review, critically examine, analyse and comment on the major points of a subject in an organised manner

University Study Skills
Academic Writing

What is academic writing?

'Academic writing' is a specific way of writing when communicating research or discussing an argument/point of view. It has a logical structure, and it uses formal language. There is a particular tone, 'voice' and style to the language. Unlike creative or narrative writing, academic writing will also use different sources of information to support what is being said.

The language of academic writing: do's and don'ts

- Do use words you know the meaning of and are confident using, it doesn't have to be complicated to be clear!
- Do not use contractions; don't, can't, doesn't, it'd. Do write out fully; do not, cannot, does not, it would.
- Do not use colloquialisms- this is 'writing as you speak'. Examples include misuse of the words 'literally' or 'basically', common phrases, such 'like chalk and cheese'.
- Do not use slang or jargon. For example, 'awks', 'lit', 'woke'.

Expressing your opinion in academic writing

In academic writing, it is best practice to express an opinion without writing in the first person, which can often be challenging. Always bear in mind that your work should read like a voice that is guided by the evidence and not basic personal intuition.

Therefore, rather than saying 'In my opinion, this proves that', you can express the outcome of your reasoning in other ways:

- 'This indicates that…';
- 'The aforementioned problems in Smith's argument reveal that…';
- 'Such weaknesses ultimately mean that…', and so on.

Signposting

Signposting guides your reader through different sections of your writing. It lets those who read your writing know what is being discussed and why, and when your piece is shifting from one part to another. This is crucial to for clear communication with your audience.

Signposting stems for a paragraph which expands upon a previous idea	Signposting stems for a paragraph which offers a contrasting view
Building on from the idea that … (mention previous idea), this section illustrates that … (introduce your new idea).	However, another angle on this debate suggests that … (introduce your contrasting idea)
To further understand the role of …(your topic or your previous idea) this section explores the idea that … (introduce your new idea)	In contrast to evidence which presents the view that … (mention your previous idea) an alternative perspective illustrates that …
Another line of thought on … (your topic or your previous idea) demonstrates that …	However, not all research shows that … (mention your previous idea). Some evidence agrees that …

University Study Skills
Referencing

What is a reference or referencing?

A reference is just a note in your assignment that tells your reader where particular ideas, information or opinions that you have used from another source has come from. It can be done through 'citations' or a 'bibliography'.

When you get to university, you will need to include references in the assignments that you write. As well as being academic good practice, referencing is very 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.** You must be careful to reference your sources correctly.

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.

University Study Skills
Referencing

Is it a source worth citing?

Question your sources before referencing using these tips:

Currency: the timelines of the information

- When was it published or posted? Has it been revised or updated? Does your topic require current information, or will older sources work as well?

Relevancy: the importance of the information for your needs

- Does the information relate to your topic or answer your question? Who is the intended audience? Have you looked at a variety of sources?

Authority: the source of the information

- Who is the author/publisher/source/sponsor? What are the author's credentials? Is the author qualified to write on the topic?

Accuracy: the reliability and correctness of the source

- Is the information supported by evidence? Has the information been reviewed or refereed? Can you verify whether it is a personal or professional source? Are there errors?

Purpose: the reason the information exists

- Does the author make the intensions/ purpose clear? Is the information fact opinion or propaganda? Are there are biases? Does the viewpoint appear objective?

University Study Skills
Referencing

How do I reference?

- There are a number of different ways of referencing, but most universities use what is called the Harvard Referencing Style. Speak with your tutor about which style they want you to use, because the most important thing is you remain consistent!

- The two main aspects of referencing you need to be aware of are:

1. In-text citations

- These are used when directly quoting a source. They are located in the body of the work, after you have referred to your source in your writing. They contain the surname of the author of the source and the year it was published in brackets.
    - E.g. *Daisy describes her hopes for her infant daughter, stating "I hope she'll be a fool—that's the best thing a girl can be in this world, a beautiful little fool."* ***(Fitzgerald, 2004)***.

2. Bibliography

- This is a list of all the sources you have referenced 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.

- Websites – Author (if possible), *title of the web page*, 'Available at:' website address, [Accessed: date you accessed it].
    - E.g. *'How did so many soldiers survive the trenches?'*, Available at: http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [Accessed: 11 July 2019].

- Books – Author surname, author first initial, (year published), *title of book*, publisher
    - E.g. Dubner S. and Levitt, S., (2007) *Freakonomics: A Rogue Economist Explores the Hidden Side of Everything,* Penguin Books

- Articles – Author, *'title of the article'*, 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'*, The Guardian Newspaper, 10 July 2014.

University Study Skills
Evaluating your sources

Knowing about the different types of sources and what makes them worth using is important for academic work.

When doing research you will come across a lot of information from different types of sources. How do you decide which source to use? From newspaper articles to books to tweets, this provides a brief description of each type of source, and breaks down the factors to consider when selecting a source.

A platform for millions of very short messages on a variety of topics.

Blogs (e.g. Tumbler) are an avenue for sharing both developed and unpublished ideas and interests with a niche community.

A collection of millions of educational, inspirational, eye-opening and entertaining videos.

A reporting and recording of cultural and political happenings that keeps the general public informed. Opinions and public commentaries can also be included.

A collection of analytics reports that outline the objectives, background, methods, results and limitations of new research written for and by scholars in a niche field.

The information presented is supported by clearly identified sources. Sometimes each chapter has a different author.

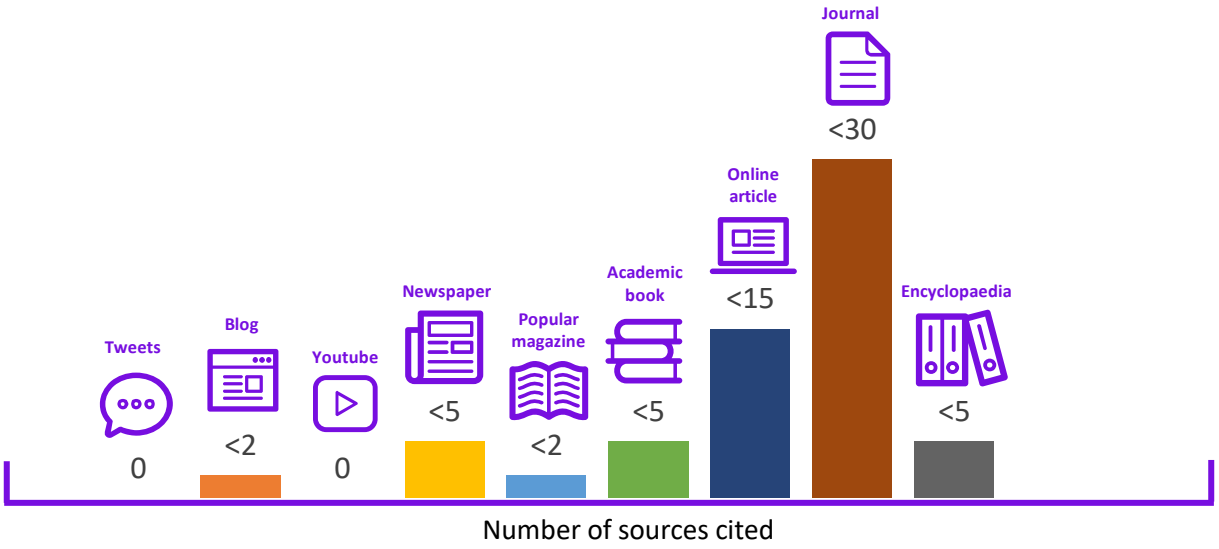
Books or online – giving information on many different subjects. Some are intended as an entry point into research, some provide detailed information and onwards references.

A glossy compilation of stories with unique themes intended for specific interests.

University Study Skills
Evaluating your sources

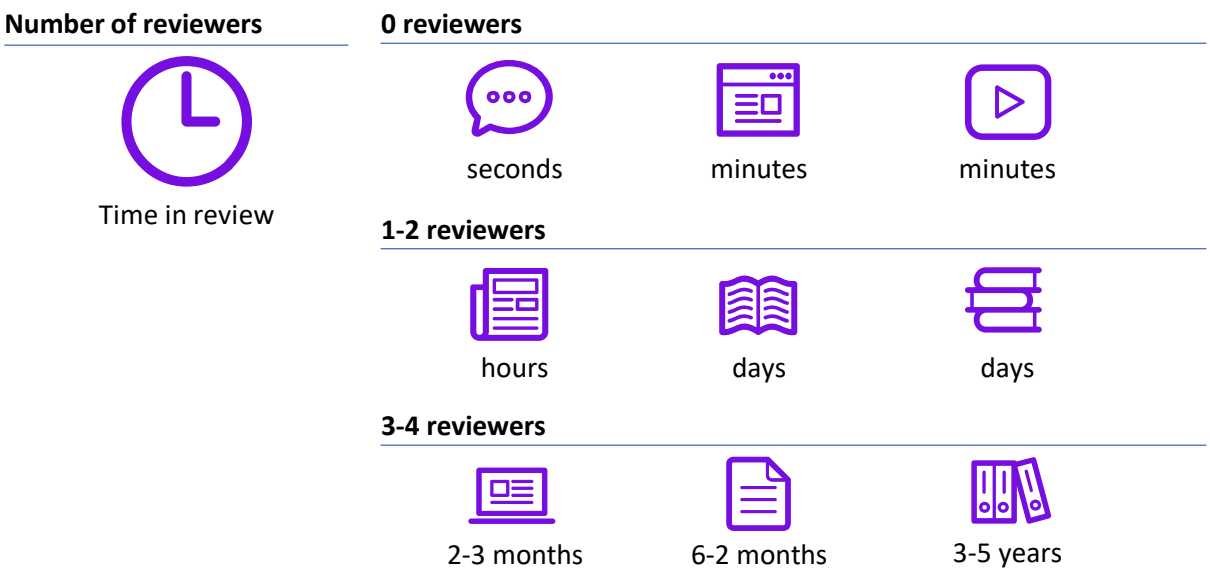
Number of outside sources

When an author used many outside sources into their writing, they demonstrate familiarity with ideas beyond their own. As more unique viewpoints are pulled into a source, it becomes more comprehensive and reliable. This shows the typical number of outside sources used in each publication.



Degree of review before a source is published

Two factors contribute to the amount of inspection that a source receives before it might be published: the number of reviewers fact-checking the written ideas, and the total time spent by reviewers as they fact-check. The more people involved in the review process and the longer the review process takes, the more credible the source is likely to be.



University Guidance
What next?

University Guidance

Different people go to university for different reasons. You might have a particular job in mind or just want to study a subject you are passionate about. Whatever your motivations, going to university can help improve your career prospects, as well as develop your confidence, independence and academic skills.

Choosing a course and university

Choosing the right course to study is an important decision so make sure you take time to research the different options available to you. Here are some top tips:

- ✓ You don't have to choose a course which you have already studied, there are lots of courses which don't require prior knowledge of the subject. You can apply skills gained from school studies to a new field.
- ✓ The same subject can be taught very differently depending on the course and university you choose. Take a look at university websites to find out more about the course content, teaching styles and assessment types.
- ✓ When choosing a university, think about what other factors are important to you. Do you want to study at a campus university or be based in a city centre? What accommodation options are there? Does the university have facilities for any extracurricular activities you're involved in?
- ✓ To research your options, have a look at university prospectuses and websites, as well as seeing if there are opportunities to speak to current students who can give you a real insight in to what life is like there.

Insight into: University of Reading

The author of this coursebook attends the University of Reading.

The University of Reading runs a large number of sessions to help find out more about the process of applying to university as well as taster sessions and Open Online Courses in a number of different subjects. To find out more, visit: www.reading.ac.uk/virtual-events.

Chat to current University of Reading students via Unibuddy and get their views on what university life is like!

University Guidance
What next?

Exploring Careers and Subject Options

- ✓ Find job descriptions, salaries and hours, routes into different careers, and more at https://www.startprofile.com/
- ✓ Research career and study choices, and see videos of those who have pursued various routes at http://www.careerpilot.org.uk/
- ✓ See videos about what it's like to work in different jobs and for different organisations at https://www.careersbox.co.uk/
- ✓ Find out what different degrees could lead to, how to choose the right course for you, and how to apply for courses and student finance at https://www.prospects.ac.uk/
- ✓ Explore job descriptions and career options, and contact careers advisers at https://nationalcareersservice.direct.gov.uk/
- ✓ Discover which subjects and qualifications (not just A levels) lead to different degrees, and what careers these degrees can lead to, at http://www.russellgroup.ac.uk/media/5457/informed-choices-2016.pdf

Comparing Universities

Use our platform ThinkUni.org to take a short quiz about your preferences and interests to find out which universities might be a great fit for you.

Other popular resources:

- ✓ https://www.ucas.com/
- ✓ https://www.whatuni.com/
- ✓ http://unistats.direct.gov.uk/
- ✓ https://www.thecompleteuniversityguide.co.uk/
- ✓ https://www.opendays.com/

University Guidance
What next?

UCAS and the university application process

All applications for UK degree programmes are made through UCAS. There is lots of information on the UCAS website to guide you through the process and what you need to do at each stage.

Apply

- Applications **open in September** the year before you plan to start university.
- You can apply for up to **five courses.**
- The deadline for most courses is **15 January**, though there is an earlier deadline of **15 October** for Oxford and Cambridge, medicine, veterinary medicine/science and dentistry.

Decisions

- Some courses may require an interview, portfolio or admissions test in addition to UCAS application. Check individual university websites details.
- Check UCAS Track which will be updated with decisions from the universities you have applied for and to see your deadline for replying to any offers.
- You should choose a firm (or first) choice university and an insurance choice. If you already have your exam results or a university thinks your application is particularly strong, you might receive an **unconditional offer**.

Results

- If you're holding a conditional offer then you will need to wait until you receive your exam results to have your place confirmed.
- Clearing & Adjustment allows you to apply to courses which still have vacancies if you didn't meet the conditions of your offer, have changed your mind about what or where you want to study, or have met and exceeded the conditions of your offer and would like to look at alternate options.

Personal statements

A really important part of your application is the personal statement. The personal statement gives you the opportunity to tell universities why they should offer you a place.

Here a few top tips for making your personal statement stand out:

- You can only submit one personal statement so it's important that you are consistent in your course choices. Make sure you have done your research to show your understanding of the subject area and passion for it.
- Start by brainstorming all your skills, experience and attributes. Once you have everything written down, you can begin to be selective – you only have 47 lines so won't be able to include everything.
- The ABC method: action, benefit and course can be a useful way to help demonstrate your relevant experience and how it applies to the course you're applying for.

University Guidance
What next?

Personal Statement do's and don'ts

Read the tips below from real life professors and admissions staff in university Biology and Psychology departments, on the 'do's' and 'don'ts' of what to include in your personal statement:

Biology

- Tell us why you want to study Biology
- What area of Biology fascinates you? I.e., ecosystems
- Demonstrate your interest by telling us what you have recently read, watched or listened to and how they helped your understanding of Biology
- What activities or practical work have you completed which helped to develop your lab-based skills?
- Describe how your school or individual work has equipped you with the necessary knowledge and ability to be a successful Biology student.

Chemistry

- Tell us why you wish to study Chemistry. Are there any topics you have studied that have interested you and topics that you are looking forward to studying?
- What drives your interest in Chemistry; has there been any relevant news/current affairs or new research which have made you wish to learn more about chemistry?
- How will gaining Chemistry degree might help you in your career aspirations?
- Try to demonstrate a wider understanding of the subject through reading or attending events/lectures. Add any university experience that you have gained through summer schools or day visits; talk about what you got out of the experience.

Further useful resources

Be sure you know what you'll need to do to apply to university in the UK:

- ✓ Key dates and deadlines: www.access-ed.ngo/timelines-for-applying-to-university
- ✓ Get tutor advice on writing a UCAS personal statement at www.access-ed.ngo/writing-your-ucas-personal-statement
- ✓ An easy template to start practising your personal statement: https://www.ucas.com/sites/default/files/ucas-personal-statement-worksheet.pdf
- ✓ Untangle UCAS terminology at https://www.ucas.com/corporate/about-us/who-we-are/ucas-terms-explained
- ✓ Discover more about the application process including when to apply and how to fill in your application on the UCAS website.
- ✓ Read more useful advice about what to include in your personal statement on UCAS, the Complete University Guide and The Student Room.
- ✓ Attend one of our virtual sessions to find out more about applying and personal statements.

Subject Guidance

Biological Sciences at University

- ✓ Biological sciences encompass a broad area of specialist subjects linked to the study of living organisms and the science behind 'life'
- ✓ You will learn about cutting edge research working towards understanding what the building blocks of life are.
- ✓ You can find out more about different courses and entry requirements by exploring the UCAS Biological Sciences Guide online: https://www.ucas.com/undergraduate/subject-guide-list/biological-sciences
- ✓ You can find out more about the different careers by exploring the UCAS Biological Sciences Careers online: https://www.ucas.com/job-subjects/biology

www.researchbasedcurricula.com

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