ReadMe (The Manual starts at page 5)

Aim

Knowledge is one of the most valuable assets of each iGEM team and, thus, the best possible way to help other iGEM teams. Complementary expertise and newly acquired information can help all teams improve their own project. Despite the competitive element of iGEM, the underlying goal is to create something useful and good for society and therefore, we want to propose this collaborative manual to help each other future teams create awesome projects.

The aim of this **collaborative manual** is to provide best practices, guidelines, standards and useful tips for future and current iGEM team and iGEMers working on **biosensors** that detect micro/macro molecules, design detection systems and/or create test kits.

Long story short we want to make a manual that stylistically is a halfway between wikipedia and wikihow.

- https://en.wikipedia.org/wiki/Spinach_aptamer
- https://www.wikihow.com/Balance-a-Centrifuge

Exchanging information in the form of this collaborative manual enables all teams involved to help shape each other's projects and **mutually benefit** from the expertise the other teams have acquired throughout their hard work. Besides the obvious practical benefits, this mutually beneficial collaboration is also a **criteria for the iGEM gold medal**.

Your contribution

It is completely up to you what you write but make sure that is relevant or useful for other iGEMers.Your contribution can be specific to your project but should provide value to all teams collaborating on the manual in the context of 'biosensors'. To avoid redundancies and repetitions, please have a brief read of the other team's contributions (at least the title) in the section you want to contribute to.

Please note you do not have to add an additional topic. If you feel you can provide additional information to a topic covered by another team, go ahead. Just make sure to link it logically to the previous contribution. Otherwise just add a new topic with a new headline.

Also, please just make sure the format is consistent with previous contributions (fond, style, size etc.)

Suggestions

- We encourage to use step by step examples or case studies
- We encourage you to use a writing style that is easy understandable to a wider public
- We encourage to share first-hand experience about issues you faced during the project
- We encourage you to use simple images and diagrams
- We encourage you to use your own words avoiding copy-pasting works of others.

- Most of the content should be tailored for projects that apply detection/sensing.

Rules
This is a collaborative endeavour please respect the work of others.
Don't delete or change the work of others without their consent.
Don't use content that is not yours without citing the author.
Don't put sensitive data e.g. (your name, your address etc.)

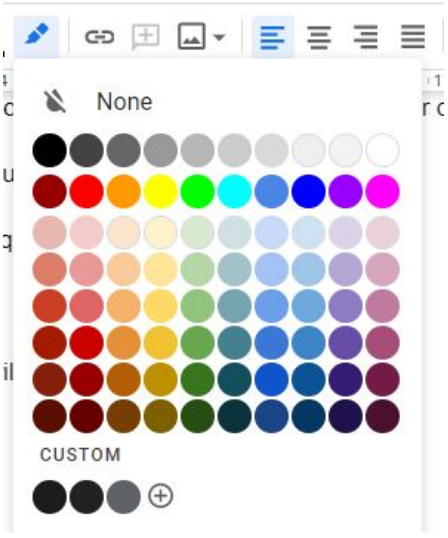
Additional
Edinburgh's iGEM team does not take responsibility for the content or data published by you and by others in this document.
Your participation is voluntary and you are aware that the document is accessible to the public.

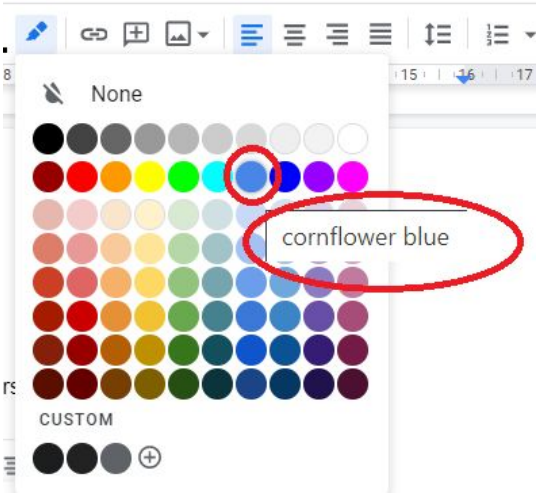
The Edinburgh's team will host a regular Zoom meeting each Saturday (1PM 13:00 UTC+1 hour) to get to know each other, organise the work and answer questions.
https://ed-ac-uk.zoom.us/j/2979483604 Meeting ID: 297 948 3604 Passcode: Collab2020

Copies of the documents will be periodically saved as back-ups.
For any enquiries or request email: edigem2020@gmail.com

How to contribute:

1. Choose a colour that will represent your university.





2. Write the colour alongside the name of the colour of your institution in this table

Check if someone already took the colour you selected*

University of Edinburgh	Cornflower blue
Uppsala University	Light green 1
Luiss	Dark magenta 2
University of newcastle	Hot pink
University of Tuebingen	Yellow

From now on, you will highlight what you write with the colour that reflect your institution

3. Choose/find a theme or thematic area e.g **Human practices → Environmental**

At the end of this tutorial check the list of themes.
If the list doesn't satisfy you, feel free to improve it.

Lab work:

---How to organise lab work

Modelling

Human practices

Ethical

---- Best practices for data collection

Safety

Environmental

---- How to make a life cycle analysis of your biosensor

Inclusiveness

Regulations and laws

---- How to apply for a patent

---- FDA guidance on false-positive or false-negative result

Business

Users

Markets

Economics

Funding

-- How to raise money for your project

4. Create a title for your contribution and insert in the list

Human practices

Ethical

---- Best practices for data collection

Safety

Environmental

---- How to make a life cycle analysis of your biosensor

Inclusiveness

Regulations and laws

---- How to apply for a patent

---- FDA guidance on false-positive or false-negative result

5. Highlight the text and put the name of the institution

Human practices

Ethical

---- Best practices for data collection

Safety

Environmental

---- How to make a life cycle analysis of your biosensor (University of Edinburgh)

6. Go to the last page of this document and start writing your contribution

The title of each section/contribution is 14 points and bold
Please when possible put sources and external hyperlinks

How to make a life cycle analysis of your biosensor

" **Life cycle analysis** (LCA) is a method used to evaluate the environmental impact of a product through its **life cycle** encompassing extraction and processing of the raw materials, manufacturing, distribution, use, recycling, and final disposal."

Mehmet Ali Ilgin, Surendra M. Gupta, Environmentally conscious manufacturing and product recovery (ECMPRO): A review of the state of the art, Journal of Environmental Management, Volume 91, Issue 3, 2010,

The steps of a life cycle analysis of your products are…..
You can use a simplified version of the LCA ….. the main benefits are….

……making your biosensor degradable by using…. would reduce its carbon footprint X times given ….

Collaborative Manual Index

List of topics and themes (feel free to improve the list)

A) Lab work:

A1) Organisation and planning

A2) Social distancing on the bench

-Implementation of safety rules in the lab and adjacent facilities(UoU)

A3) Challenges

A4) Testing

--- Lab Tests for biosensors(UoE)

B) Modelling & Design

B1) Input

B2) Processing

--- Determining the structure of peptide-based biosensors (University of Tuebingen)

B3) Output

--- list of fluorescent RNA aptamers (UoE)

--- good alternatives to fluorescent signals (UoE)

--- how to make your detection quantitative or qualitative (UoE)

B4) Programming

--- list of good programming languages for synthetic biology

--- How to make an ODE model of your biosensor

--- generic example code for modelling

C) Human practices

C1) Applications

---- Generic Targets for Biosensors (UoE)

--- User-led approach on biosensors (UoE)

C3) Ethical

---- Best practices for data collection

C3) Generic Uncategorised content

---- Macro analysis for your projects using PESTEL (UoE)

---- Micro analysis of your projects using Porter's competitive forces (UoE)

C4) Safety

---- IRCG Framework for synthetic biology (UoE)

C5) Environmental

---- How to make a life cycle analysis of your biosensor

---- Crop Disease management and biosensors (University of Edinburgh)

---- Guidelines for biosensors to use in agriculture (University of Edinburgh)

C6) Inclusiveness

--- How to make your biosensor inclusive to visual impaired people **(University of Edinburgh)**

C7) Regulations and laws

---- How to apply for a patent

---- FDA guidance on false-positive or false-negative results

--- List of regulations in place for biosensors used for water testing (UoE)

D) Business & Economics

D1) Users

D2) Markets

D3) Funding

-- How to raise money for your project

D4) Equipment

-- Useful websites for purchasing equipment

D5) Entrepreneurship

-- How to make a business plan

E) Extra

-- How to make an impactful presentation

-- How to make a successful 5 minute/elevator pitch (UoU)

A)

A1) Organisation and Planning

Conducting a long-term scientific project like iGEM requires a high level of organization, flexibility and tolerance for frustration. Due to specific local and personal circumstances, each project faces different challenges during their work. Nevertheless, there are a few ground rules and advice that can make the work easier and the iGEM experience even more enjoyable.

1) **Stick to your meeting schedule. Even if there are not a lot of things to discuss sometimes, it is important to make your group meetings a habit. A weekly meeting for the whole team is a solid start.**
2) **Divide meetings into subgroups if necessary. Some intense, detailed discussions will always be necessary: but not always for the whole team. Additional weekly/monthly/… meetings for subgroups like Wetlab, Drylab or Finance can be very useful and time-saving.**
3) **Make sure the team members and the team are reachable. Regularly check your team's mail or social media accounts so that you are always up to date.**
4) **Make meetings fun! Include memes or funny pictures of your current difficulties into meeting presentations, and try to meet in person in less formal environments. This serves as teambuilding and will be very beneficial for your work as a group!**
5) **Regularly check the iGEM Homepage for updates, releases and helpful information.**
6) **Plan ahead. This might sound trivial but is EXTREMELY important! This concerns all areas of your project. Some helpful questions might be:**
    - **What machines/equipment do we need? Does the laboratory contain them? Can we lend them from somewhere? Where can we order them?**
    - **When do we want to have finished step x of the project?**
    - **Who are possible sponsors and how can we contact them? Until when will we need to have money acquired?**
    - **How long will delivery take if we order something?**
    - **How much time do we want to spend on the wiki and uploading results? When should we stop lab work in order to properly analyze and present our data?**
7) **Think of Plan B and C. In every project, there will be predictable and unpredictable obstacles, and you should be prepared for these scenarios as much as you can. Some helpful questions might be:**
    - **What can we do if step x in our experiments does not work?**

- **What if step x takes longer than we thought?**
- **Who can take over task x if the primarily responsible person becomes unavailable?**

8) **Make a finance plan and keep updating it. You should always be aware of your current budget and what you are going to spend it on.**
9) **Stay in touch with the world! By being active on social media, you can get the attention of other teams for possible collaborations, sponsors, new team members or others who could contribute to your success!**
10) **Reach out for help and expertise! There are many ways to get assistance or guidance for your work both within the iGEM community as well as locally. Don't be afraid to approach experts, scientific groups or students, most of them will be happy to help.**

A2) Social distancing in the bench

Safety rules for laboratory work have been actively enforced and implemented since the decade of 1990 (1). Nevertheless this year, as in many other spaces, social distancing took a more relevant position in order to prevent outbreaks of COVID-19 disease in the working place.

One of the main aspects of social distancing is "Physical distancing", which according to the World Health Organization means "being physically apart". They recommend at least 1 meter between people (2).

Measures to be taken:

- *Limit the maximum amount of people that can be in the same room (lab, culture/cold rooms, lunch room etc) according to the surface available. Remove chairs to enforce the rule compliance*
- *Follow GLP rules.*
- *Install physical dividers between workplaces, if possible transparent to ease communication. Typically 1 or 2 per bench depending on laboratory dimensions.*
- *Wear a mask (1). Nevertheless this point might vary depending on the national/regional regulations of your emplacement.*

1. *https://www.fishersci.com/us/en/scientific-products/publications/lab-reporter/2018/issue-3/evolution-of-laboratory-safety-a-cautionary-tale.html. 30/09/2020 19.13*
2. *https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-coronaviruses. 30/09/2020 19.18*

A3) challenges

Challenges occurred during construction of Biosensor.

When ordering biosensor construct. DNA synthesis company was unable to make entire construct, but was able to make transcription factors and

fluorescent proteins. Binding regions were amplified out of ADP1, which contained high repeat regions and A/T rich. This caused non-specific binding and multiple bands and smear within gel lanes. This caused impure solutions which resulted in difficulty joining the construct via overlap extension PCR.

Thus, needed to revaluate the annealing temperature of primers from the suggestion from DNA synthesis company by performing a gradient PCR. Discovered different annealing temperatures for primers which resulted in less smearing and unwanted bands in gel lanes. Using traditional overlap extension PCR procedures was having a low rate of success in joining DNA fragments together. Utilised optimised overlap extension PCR protocol(reference) and had much higher success rate.

A4)

Lab tests for biosensors(UoE)

Extract from:
" Regulatory and Validation Issues for Biosensors and Related Bioanalytical Technologies " Nikolay V. Sergeev,1 Keith E. Herold2 and Avraham Rasooly1,3

WORK IN PROGRESS:

***Accuracy** can be verified by analyzing a large number of samples and comparing the measured values with the true (known) or accepted reference values obtained with a "gold standard method".*

***The precision** is calculated as a standard deviation (SD) or a coefficient of variation (CV%), which expresses the SD as a percentage of the mean value of the replicate measurements. The precision of a bioanalytical procedure is usually verified in a series of individual measurements of an analyte "when the analytical procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix".3*

***Repeatability** should be tested by a minimum of five determinations at three different concentrations (low, medium, and high) in the range of expected concentrations.3*

*Repeatability is determined by measurements performed in one laboratory by the same operator using the same equipment on the same day (also called **within-day or intraday precision**)*

Reproducibility *(also called the interlaboratory precision) is defined as the measured precision in multiple measurements obtained by different laboratories (different operators, equipment, and laboratories).*

Determination of "limit of detection (LoD)," "lower LoD," or "detection limit."*8 In these cases the* ***sensitivity*** *of a biosensor system generally corresponds to the LoD and is understood to be the minimal detectable analyte concentration typically given in units of particles or mass per unit volume (particles/liter or mass/liter).*

tests is the ability of the test system to detect the presence of the target analyte in samples where it is actually present at concentrations equal to or exceeding the LoD.

that the device must not only be capable of detection of low levels of the intended analyte ***(sensitivity)*** *but also should be able to selectively differentiate between target and nontarget analytes* ***(specificity****).*

Determine ***"limit of blank" (LoB), LoQ, "lower end of the measuring range" (LMR), and "lower limit of linear range" (LLR).*** *of LoB is taken as a basis which then is adjusted by a factor of 10 times the SD to estimate the LoQ and a factor of 2 or 3 to estimate the LoD. However, for clinical tests it is recommended that one follow the approach described by the ISO defining LoD in relation to stated levels of Type I and II errors14 (also called α and β errors in CLSI guidelines8).*

The evaluation of the test linearity *Furthermore, for a quantitative bioanalytical method (i.e., real-time qPCR), it is impossible to accurately interpolate between points unless the functional form of the results is known and the simplest functional form is a linear relationship.*

the ***"upper limit of the linear range" (ULR).*** *The test linearity can be verified with a calibration (standard) curve generated with six to eight nonzero standard samples prepared in the same matrix as the samples in the intended study.3*

The ***linear range*** *of an analytical method is defined as the span of analyte concentrations for which the system output results are directly proportional (with stated trueness and precision) to the input analyte levels. The* ***measuring range*** *(also referred to as the reportable range or working range15) is similar to the linear range but without the requirement for the direct proportionality.*

safety and effectiveness.
……..
In cases such as microbial identification assays, the goal is to define "positives" and "negatives" with a high degree of certainty because mistaken diagnosis can lead to adverse health effects due to inappropriate treatment.

“ Regulatory and Validation Issues for Biosensors and Related Bioanalytical Technologies “ Nikolay V. Sergeev,1 Keith E. Herold2 and Avraham Rasooly1,3

B)

B2) Processing

Determining the structure of peptide-based biosensors (University of Tuebingen)

When experimenting with biosensors, their three dimensional structure is not necessarily known. Especially when we modify the sensors, for example to improve its binding strength, binding specificity or capacity, the structure can change as well, either willingly or not. Knowing about the structure can be important to characterize different parts of the biosensor or to determine whether there is an intact binding site. We can also base modification on the knowledge about structure, respectively the knowledge about which parts are in proximity and thus probably interact.

For peptide-based biosensors with known sequence, the structure can be determined via homology modelling, if there are (enough) known homologous with a similar sequence. These methods compare the biosensor sequence against a database of protein sequences with known structure. The structures of close matches can be used as templates. Threading is often used to find template structures in a parallelized and fast way.

If there only exists little or no knowledge about homologous proteins, *ab-initio* modelling might be necessary. In this case, a free energy minimization is performed while iterating over different possible structures for smaller parts of the sequence. (1)

Many tools for protein structure prediction use a combination of these methods. Examples for often used tools are SWISS-MODEL (2), RaptorX (3) and I-TASSER (4).

In any case, the stability of the structure needs to be tested in a molecular dynamics simulation. The structure is invalid if it bursts during a simulation of at least 50 ns. The choice of appropriate parameters and especially the force field is important at this point and can influence the result strongly. Looking for experience of researchers with a similar modelling problem is helpful.
If this first test is positive, molecular dynamics simulation can be used for further analysis of the biosensor, for instance to determine exact binding constants or for flux analyses.

An often used tool for molecular dynamics simulation is GROMACS (5).

Sources:

1) https://bioinformaticsreview.com/20171210/ab-initio-prediction-of-protein-structure-an-introduction/

2) Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46(W1), W296-W303 (2018). https://swissmodel.expasy.org/

3) Morten Källberg, Haipeng Wang, Sheng Wang, Jian Peng, Zhiyong Wang, Hui Lu & Jinbo Xu. **Template-based protein structure modeling using the RaptorX web server.** *Nature Protocols* 7, 1511–1522, 2012. http://raptorx.uchicago.edu/

4) A Roy, A Kucukural, Y Zhang. I-TASSER: a unified platform for automated protein structure and function prediction. Nature Protocols, 5: 725-738 (2010). https://zhanglab.ccmb.med.umich.edu/I-TASSER/

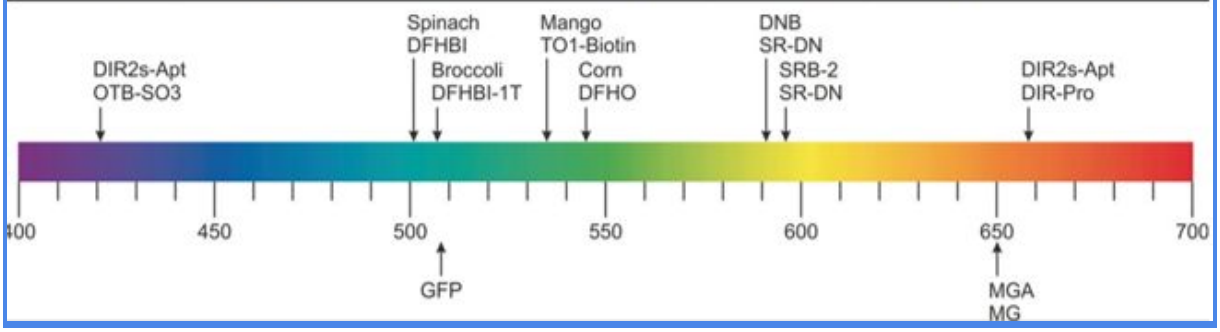
5) Abraham, Mark James, et al. "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers." *SoftwareX* 1 (2015): 19-25. http://www.gromacs.org/

B3) Output

--- *list of fluorescent RNA aptamers (UoE)*

There are a multitude of various RNA aptamers available to use as output signals for Biosensors. As opposed to the fluorescent proteins, their advantage is the faster synthesis time, therefore faster detection. The RNA aptamers are also shorter in length and do not impose a huge burden on the chassis organism that expresses them. The RNA aptamers have various temperature, cofactor or buffer requirements to work properly, therefore choosing the right aptamer for the specific application can be laborious. The figure below shows a list of some of the most used RNA aptamers, and this list is always expanding.

RNA aptamer	Fluorogen	λ Excitation/ Emission (nm)	ε Extinction coefficient (M−1 cm−1)	Φ Quantum yield	Brightness	Length (nucleotides)
eGFP		**490/508**	**55000**	**0.6**	**33000**	**N/A**
MGA	MG	630/650	150000	0.19	28500	38
Spinach	DFHBI	469/501	24300	72	17500	98
Spinach2	DFHBI	445/501	26100	0.7	18300	95
	DFHBI-1T	482/505	31000	0.94	29100	95
iSpinach	DFHBI	442/503	26100	0.98	25000	69
Broccoli	DFHBI-1T	472/507	29600	0.94	27800	49
Chili	DMHBI+	456/592	21000	0.4	8400	52
Corn	DFHO	505/545	29000	0.25	7300	2x36
Orange Broccoli	DFHO	513/562	34000	0.28	9500	60
Red Broccoli	DFHO	518/582	35000	0.34	12000	60
Mango -I	TO1-Biotin	510/535	77500	0.14	10900	29
	TO3	637/658	9300	N/A	N/A	29
Mango -II	TO1	510/535	77500	0.22	16000	30
Mango-III	TO1	510/535	77500	0.56	43000	31
Mango-IV	TO1	510/535	77500	0.41	32000	30
DIR2s-Apt	DIR-Pro	658	164000	0.33	54100	57
DIR2s-Apt	OTB-SO3	421	73000	0.51	37200	57
SRB-2	SR-DN	596	N/A	0.65	N/A	54
DNB	SR-DN	591	50300	0.98	49300	75



An image representing some of the available RNA aptamers and their characteristics. The top part of the figure is a table with the RNA aptamers and their fluorogens that increases their fluorescence. The λ excitation and emission wavelength in nanometers are displayed, ε extinction coefficient in M^{-1} cm^{-1}, Φ quantum yield, their brightness (Φ*ε) and length in nucleotides. The first row is the enhanced Green Fluorescent Protein (eGFP) highlighted in red, it's the "gold standard" for fluorescence in biology. The Corn RNA aptamer is a homodimer (2x36). The lower part of the image is the RNA aptamers with their fluorogens placed on the visible spectrum (400 to 700nm) to showcase their colour. Dimethyl indole red (DIR), 3,5-difluoro-4-hydroxybenzylidiene imidazolinone (DFHBI), 3,5-difluoro-4-hydroxybenzylidene imidazolinone 1 trifluoroethyl (DFHBI-1T), 3,5-difluoro-4-hydroxybenzylidene imidazolinone-2-oxime (DFHO), malachite green (MG), oxazole thiazole blue (OTB), sulforhodamine-dinitroaniline (SR-DN), thiazole orange 1 (3)(TO-1 (3)), Source: modified from (Bouhedda et al, 2018; Truong & Ferré-D'Amaré, 2019).

C1) Application

Generic Targets for biosensors (University of Edinburgh)

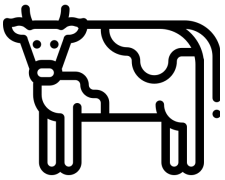
A biosensor could have different applications and targets that could be divided in categories.

The first category of targets are diseases.

Diseases could be divided into three broad categories: human diseases, animal diseases and plant diseases.

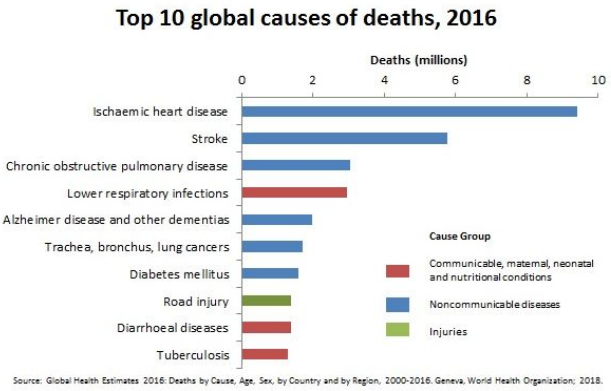
TARGET: DISEASES

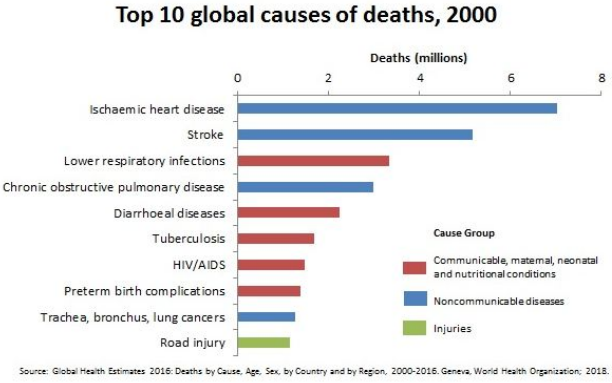
HUMAN (12420) **ANIMAL** **PLANT**



Human diseases:
While the creation of point of care (PoC) diagnostics are relevant for any kind of disease, diagnostics remain particularly effective for infectious diseases.
Here's a list of human infectious diseases: https://health.ri.gov/diseases/infectious/

Moreover, since the global causes of death are attributed to an handful of diseases and conditions, biosensors that could help preventing and detecting these health conditions and diseases have a high social impact.





Lastly there are over 10,000 of human diseases that are known to be related to our genes. While genetic tests made in laboratories are preferred, this does not take away the fact that it is critical for the rise of personalised medicine to develop detection methods and biosensors that target human genes. https://www.who.int/genomics/public/geneticdiseases/en/index2.html

Animal diseases: work in progress
Human diseases: work in progress

The second category of potential targets are chemicals and pollutants.

Biosensors are very useful in detecting dangerous chemicals and pollutants. While qualitative biosensors could be helpful to determine the presence of extremely dangerous chemicals and pollutants, the development of quantitative biosensors should be preferred since some chemicals and pollutants become dangerous only at certain thresholds.

(The results of quantitative biosensors should be easy to read and understand. For more information see “How to make your biosensor inclusive to visual impaired” and “ how to make your detection quantitative or qualitative”)

Here’s a comprehensive list of extremely dangerous chemicals:

https://en.wikipedia.org/wiki/EPA_list_of_extremely_hazardous_substances

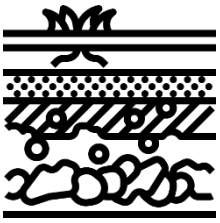
TARGET: HAZARD CHEMICALS AND POLLUTANTS

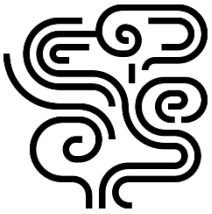
Putting aside PoC tests when considering a target for your bionsensor regardless if it is organic or inorganic it is important to consider on what substrate, layer or surface you want to detect your target.

For biosensors that are focused on environmental issues there are four main substrates/layers/surfaces to consider before developing your biosensor.

Each substrate/layer/surface poses specific design challenges.

FOOD **WATER** **SOIL** **AIR**





The third category of potential targets are the ones that replace traditional sensors and chemical and mechanical detection methods.

In this regard, traditional sensors are used in different industries for various purposes. These traditional sensors result very efficiently but often are not eco-friendly or degradable on the contrary these could generate high levels of e-waste and pollution. Therefore in a not so distant future sustainable biosensors could.

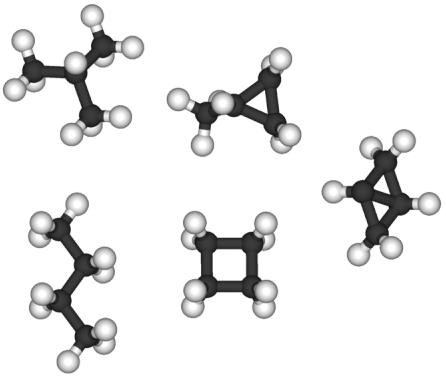
Examples of biosensors that could have industrial applications are the ones that are efficiency related, (for example the ones that describe when a process is not efficient/optimal or when is optimal) or the ones that could be used for ongoing monitoring (the ones that measure corrosion/oxidation, temperature, photosensitivity, radiation etc.)

Lastly biosensors that detect precious and non-precious metal could also have industrial applications

TARGET: INDUSTRIAL/SCIENTIFIC APPLICATIONS

ELECTROMAGNETIC **TEMPERATURE** **ETC.**

PRECIOUS/ NOT PRECIOUS METALS



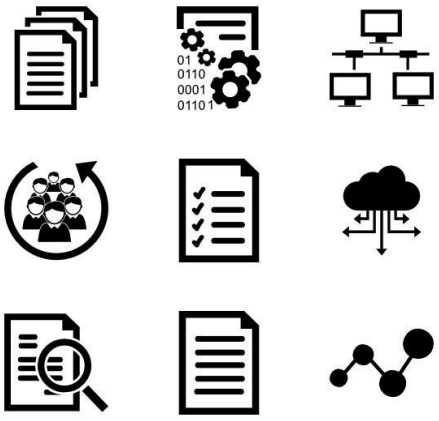
User-led approach for biosensors (University of Edinburgh)

The only purpose of a sensor or biosensor is to gather data. Therefore we should ask ourselves: Why are we collecting data? For who exactly?

To have a user-led approach in designing and developing biosensors, these questions should be followed by other questions: What data is useful? Why is it useful? Who is going to benefit from the data? Who is the user and final-user of the data gathered?

In fact a good biosensor not only provides data but it provides essential and critical data for a given user. If the data is not essential to the user the biosensor has only a relative societal impact.

WHAT DATA IS USEFUL, ESSENTIAL FOR THE USER?



Data could still be essential even if the user doesn't recognise the data to be essential. In fact the user could have an altered perception of the relevance of the data that has been gathered by a bionsensor, in that case the problem often relies on how the data and its importance have been communicated to the user and end-users.

A different case is when the biosensor produces data that previous sensor and biosensor never detected, in that case even if it appears that there are no applications or markets for it there may be a group of users that could benefit. In this case when the user is not aware of the existence of a new kind of data or service that could benefit him/her, we talk about **unknown needs.**

Usually the developer of the biosensors is the one that has to start an exploratory process to identify unknown needs.

THERE IS DATA THAT THE USER IS NOT AWARE OF THE IMPORTANCE? (UNKNOWN NEEDS)

Regardless of known and unknown needs you should always ask yourself: Why should a user use our biosensor?

Obviously as a scientist or developer you could think about thousands of reasons why your biosensor or test kit is amazing and wonderful, but disappointingly your opinion matters only to you.

Since we are talking about a user-led approach to biosensors the only opinions that matters are the ones of the users and end-users. In fact, scientists and synthetic biologists when developing and producing a biosensor should be prepared to face users that have different needs and that don't understand basic biology.

Most cases the end user is less skilled and knowledgeable than the producer and this is ok (you don't have to be a computer expert to use a computer and not everyone has to be a biologist) .

Again: Why should a user use our biosensor?

WHY AN "USER" SHOULD USE OUR BIOSENSOR?

FASTER? CHEAPER? EFFICIENT? SAFER?

DO MULTIPLE THINGS AT ONCE?

To answer the question you have to formulate a Value Proposition. Generally a Value Proposition consists of a short formula/set of sentences that explain what benefits an end-user will receive by using your biosensor.

The value proposition should be clear and specific, an end-user wants to have judging parameters when evaluating a product such as a test kit.

IS BETTER? HOW MUCH BETTER? (E.G. TWO TIMES FASTER)

The value proposition not only should explain the benefits but also guides the user on how to achieve those.

Lastly remember that having a biosensor or product that is technically better than others does not guarantee future success or adoption of your biosensor.

There are elements such as public perception, regulations, markets or scalability that are independent from technical superiority.

A man forewarned is forearmed, it is your duty to account for external elements that contribute to the success or failure of your project.

TECHNICALLY BETTER ≠ SUCCESSFUL

DO NOT REPEAT MISTAKES:
PUBLIC PERCEPTION AND STAKEHOLDER ENGAGEMENT

C3) Generic Uncategorised content

Broad PESTEL Macro analysis for you projects and products (University of Edinburgh)

In order to categorize the vast sea of external factors influencing your project you can use the PESTEL analysis.

Most commonly known as PEST (political, economic, socio-cultural and technological) this analysis could have many variants that account for more parameters but for the time-being we are going to use one that also account for environmental and legal parameters:

PESTEL (political, economic, socio-cultural, technological, environmental and legal).

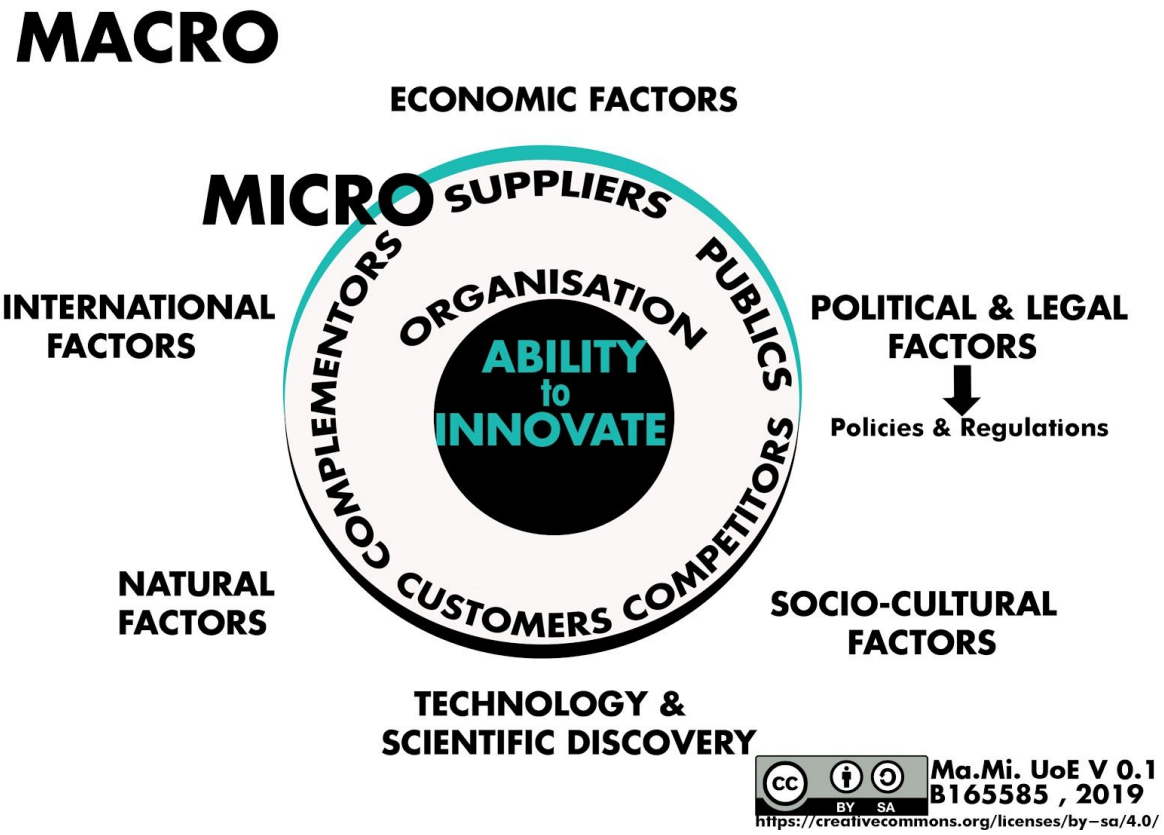
The other variants (https://en.wikipedia.org/wiki/PEST_analysis) :

- **SLEPT**, adding legal factors.
- **STEPE**, adding ecological factors.
- **STEEPLE** and **STEEPLED**, adding ethics and demographic factors (occasionally rendered as **PESTLEE**).
- **DESTEP**, adding demographic and ecological factors.
- **SPELIT**, adding legal and intercultural factors, popular in the United States since the mid-2000s.
- **PMESII-PT**, a form of environmental analysis which looks at the aspects of political, military, economic, social, information, infrastructure, physical environment and time aspects in a military context

PESTEL is a framework that helps you find the macro factors influencing and shaping your project. Macro factors are considered external from an organisation, usually you can consider the marofactors the one that you cannot control.

the framework is very useful in finding areas where you can exercise human practices

TO CONTINUE



TO CONTINUE

C4) Safety

IRCG Framework for Synthetic biology (UoE)

"Biosecurity is a strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and activities) for analysing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment."
(FAO , 2007)

In 2005 The International Risk Governance Council (IRGC) was confronted by global challenges that can be best characterized by complexity uncertainty and ambiguity, such as terrorism, nuclear weapons, new diseases and emerging new technologies. In that year the White Paper of the Council “Risk Governance: Toward an Integrative Approach (IRGC 2005)”, quickly became one of the most relevant and useful framework for both risk managers and decision makers currently facing risks.

So what is a risk? A risk is the combination of the likelihood of potential consequences and the grade of severity of events on humans and/or what humans value.

Getting into technical details, the framework have five main elements (pre appraisal, appraisal(assessment),characterization and evaluation, management, cross cutting aspects) (IRCG, 2017) that should also not be seen as sequential steps but as elements that are closely interlinked

- Pre-assessment is the process where the main actors involved start to identify the risks (early warnings and monitoring) and begin an open conversation with all the stakeholders. One key component of pre-assessment is the selection of conventions and rules to use.

- Risk assessment is the generation and gathering of knowledge in all his dimensions (economical, technological, physical, etc.) to link possible causes, consequences and concerns of the stakeholders to a specific risk.

- Risk characterisation and evaluation is the process attributing one or more characteristics (simple, complex, uncertain or ambiguous) to a risk.

- Risk management is performed after reviewing all the relevant information to decide the appropriate options to change human activities or/and natural and artificial structures to potentially prevent harm and increase benefits to humans and/or what humans value.

- Risk communication and stakeholder engagement are considered a cross-cutting aspects that are and should be present in each stage of the

IRGC framework as critical components to successful assessment and managerial activities.

Generally for synthetic biology it could be useful to distinguish the five main elements between an assessment sphere (containing risk appraisal) and management sphere (containing decision making and implementation) (Bunting et al, 2008).

TO CONTINUE

C5) Environmental

Crop Disease management and biosensors (University of Edinburgh)

A biosensor is just a part of the solution in preventing managing and eradicating a pathogen/pest.

Usually we can identify 5 main areas for the eradication of pathogens and pests that affect crops and plants.

The biosensor could be particularly useful tool only some of the 5 areas.

https://www.apsnet.org/edcenter/disimpactmngmnt/topc/Pages/PlantDisease Management.aspx

EXCLUSION

This principle is defined as any measure that prevents the introduction of a disease-causing agent (pathogen) into a region, farm, or planting

ERADICATION

This principle aims at eliminating a pathogen after it is introduced into an area but before it has become well established or widely spread. But generally is not effective over large geographic areas. Two large attempts at pathogen eradication either of these attempts was a lasting success.

PROTECTION

This principle depends on establishing a barrier between the pathogen and

the host plant or the susceptible part of the host plant. It is usually thought of as a chemical barrier, e.g., a fungicide, bactericide or nematicide, but it can also be a physical, spatial, or temporal barrier.

RESISTANCE

Use of disease-resistant plants is the ideal method to manage plant diseases, if plants of satisfactory quality and adapted to the growing region with adequate levels of durable resistance are available. The use of disease-resistant plants eliminates the need for additional efforts to reduce disease losses unless other diseases are additionally present.

INTEGRATED DISEASE MANAGEMENT

Integrated Disease Management (IDM) is a concept derived from the successful Integrated Pest Management (IPM) systems developed by entomologists for insect and mite control.

Generally who is developing a biosensor should look if there are existing kits,tests and targets on the market and understand their attributes and compare these attributes against the biosensor.

The developer/scientist should also assess what solutions are the implemented activities against the disease or pest and what solutions could be implemented to eradicate the disease or pest.

- New pesticides ?
- New plant varieties ?
- New technologies (biosensors) ?
- New practices ?
- A Combination ?

Even if checking other solutions is not directly related to the production and development of a biosensor, it should be always done since it could give the developer/scientist good insights on how to develop his/her bionsensor.

Moreover checking the implemented activities and solution is a great exercise to understand how benefits counterbalance risks.

In fact depending on the culture and the legal system, it is ok to take risks if the benefits exceed the drawbacks.

(E.g. it is ok to burn hectares of infested cultivated land and infested forest if it saves the agricultural sector of a country?)

The use of biosensors in detecting chemical pollutants in the environment ie Benzene.

Environmental pollution is one of the major threats facing the world today. Recently, a crisis unfolded in the waters surrounding the Mauritius. A ship filled with petrochemicals (chemicals derived from petroleum or natural gas) ran aground and leaked petrochemicals into the ocean, devastating sea life and causing catastrophic harm to nearby reefs (ABC, 2020).Spills such as this can have pollutants travel long distances from initial site, at different concentrations depending on currents or on terrestrial spills the slope of the land.

A biosensor for detecting harmful petrochemicals such as benzene, can be used to identify regions with high levels of contamination, to organise the most appropriate location for clean-up ie bioremediation of the soil. Thus biosensor can be a useful tool in organising bioremediation and highlighting pullulated areas and their cause.

C7) Regulations and Laws

List of regulations in place for biosensors used for water testing (UoE)

Work in progress most of regulations listed are UK based, we will other national regulations soon

Check Health and Safety at Work etc. Act 1974
Check Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677).
Check SC website http://www.rsc.org/learn-chemistry/collections/health-and-safety
Check “Safe Practices in Chemical Laboratories” and “Hazards in the Chemical Laboratory”, 1992, produced by the Royal Society of Chemistry
Check “Guidelines for Microbiological Safety”, 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee

Check: “Safety Precautions, Notes for Guidance” produced by the Public Health Laboratory Service
Check “Good Laboratory Practice” produced by the Department of Health.
Check EN ISO/IEC 17025 (2005).
Check European Union (EU) Commission Directive 2015/1787
Check ISO 11352 (2012) Water Quality: Estimation of Measurement
Check Water Framework Directive (WFD)
Check Drinking Water Directive (DWD)
Check analytical quality control (AQC)
Check ISO 17025
Check NS30 (Cheeseman and Wilson, 1989)
Check proficiency testing (PT) schemes
Check NS30 (Cheeseman and & Wilson, 1989),
Check Eurachem/CITAC Guide (Ellison and Williams, 2012)
Check International Union of Pure and Applied Chemistry (IUPAC)

Laws for use of GMO biosensor within Australia

Due to biosensors that are within a genetically modified organsism, in Austrlia must follow all guidelines outlined by OGTR. This includes physical containment ie within a lab and transported in a sealed unbreakable primary container. GMO Biosensor use must be restricted to people with the appropriate qualifications to be legally allowed to work with GMO. Due to these guidelines testing of samples with a GMO biosensor must be done in building with appropriate safety measures and sample disposed of via appropriate guidelines, ie PC2 lab, requires autoclaving of biological material.

D) Business and economics

D3) Funding

Over the last 15 years, synthetic biology startups have been funded with over 1.1 billion pounds. While the absolute number of investments has decreased, their volume has increased substantially with average fundings of 9.9 million pounds in 2018. As early stage ventures still dominate the majority of investments being made, more and more biotech entrepreneurs hope to get fundings from large VCs or individual angel investors but how exactly does early stage funding work?

The typical funding cycle goes as follows: pre-seed funding followed by Series A, Series B, Series C etc. Pre-seed funding is often made available by the founder him/herself or/and family members and friends. It is the earliest stage of funding and the funds are usually used to get the venture off the ground in the first place. Once the seed stage is passed and the startup demonstrates some traction in the form of customers, revenue and other performance indicators, Series A funding may be considered. The key players at this stage are venture capital firms as well as Angels Investors, who fund the startup for equity in the company. The transition from seed-funding to Series A funding is usually the most difficult to perform. If the company keeps or increases its momentum and traction, it may go through further stages of funding. There are several ways in which startups can get funding from VCs or angels! A popular path is through accelerators and incubators. While only some offer direct funding against equity, most provide participating teams with very valuable networking opportunities with investors. Prestigious programmes like Y combinator give the teams the opportunity to pitch to an audience of selected investors on demo day, which often leads to subsequent investments.

To understand how the investments affect the ownership of the company, there are two terms to consider. Pre-money valuation and post-money valuation. Pre-money valuation refers to the value of the company, excluding the respective investment while post-money valuation refers to the value of the company following the last rounds capital injection. Investors usually try to negotiate a low post-money valuation. The reason can be illustrated with this example: If company A is worth 500,000 EURO pre-money (without investment) and investor X invests 50,000 EUR), the post-money valuation would be 550,000 and investors X would own 10% (50,000/500,000). However, this implies investors and entrepreneur agree on pre and post-money valuation. Different valuation may, thus, influence equity of each party. Another important aspect of investment rounds to consider is dilution.

When new funding is raised in subsequent rounds, equity of current investors may be diluted to accommodate the issuance of new shares. Founders and investors usually carefully determine terms and conditions for potential dilutions.

Funding for startups can come from entirely different directions: Founders can crowdfund on popular platforms, get loans from banks, apply for grants or go the hard way of bootstrapping without outside capital.

E) Extra

E1) How to make a Successful 5 minute pitch (Uppsala University)

**Inspired by Drivhuset (A Swedish organization helping students develop entrepreneurship and ideas to eventually form businesses)*

The elevator pitch relies on that you, as the speaker, presents your idea as concisely as possible in a limited amount of time. This may be useful for presenting your project but even catching the attention of possible investors. This is important to keep in mind, as you need to steer the focus of your pitch towards what your goal is in regards to who is listening. In order to perform a successful “elevator pitch”, there is a general structure to follow.

1) Capture the interest of your listener(s)

    This can be done through a rhetorical question, metaphors, examples or humor

        E.g. Have you ever wondered what the world would look like without hunger?

        E.g. Make a similarity between your solution and something else which they can relate to.

2) Focus on the problems and needs in regards to the specific topic you will be presenting a solution for.

    This should be short.

    Clarify what the consequences of these problems or needs are and/or what are current solutions to this problem or need?

    Why is the current solution available not sufficient?

3) Create value: How are you solving this problem?

    This should be short and concise, remember who the listener is - do they know all the terminology?

    Describe your idea and how it will function as a solution

4) Present benefits with your solution

    How are you solving this problem or need you introduced earlier?

    How is this beneficial for your listener?

        E.g. If they are a Biotech company, can you bring them value through increased profits or contribution to an important cause?

5) Compare your solution to other alternatives

    How is your solution better than the alternatives, if there are any.

6) Close the pitch in a clear manner

Remember who the listener is - what do you need from them?

- *Do you want them to fund your project? Direct them to your GoFundMe/website/specific contact person*
- *Do you want them to get in touch? Tell them how to do so etc.*

To summarize, you need to have a **Hook** to capture the attention of the speaker, followed by introducing a **Problem or Need** and a specific **Solution** you can provide. Then, you present the **Benefits** of this solution and **Compare** to existing solutions and then **Close the pitch** by "telling" the listeners what you need from them.

Remember, having a presentation may be necessary but you could also engage your listeners by not having one - it could be more beneficial not having one by keeping the attention to your words rather than to what is written!

Whilst following the structure above, there are some important Do's and Don'ts to keep in mind!

Do:

- Be concise and straight to the point
- Use body language whilst speaking
  - *This enganges listeners and makes them like you more!*
  - *Stand up if you can, instead of sitting it makes you feel more free*
- Throw in a joke or two
- Speak slower than usual
- Inspire your listeners
  - Throw them off their feet as a way to stick out
  - Make the pitch alive and give it a pulse

Don't:

- Overload on information or oversell the idea
- Talk the wall
  - *Try to make eye contact/if you look above speakers it seems as if you are making eye contact*
  - *When presenting online this is easier!*
- Have too many side-effects on slides or too small text
  - *It's distracting and may seem less professional*
- Use acronyms or industry terminology - if they are not familiar with it
- Make people doubt your statements
  - *If you are clear and concise this is less likely*
- Use bad quality images
  - *It's distracting*
- Go overtime
  - *People lose interest when you speak past the time you were given*