Microbial Surface-Display

Mommy- are microbes smooth like a beach ball or rough like a tennis ball?

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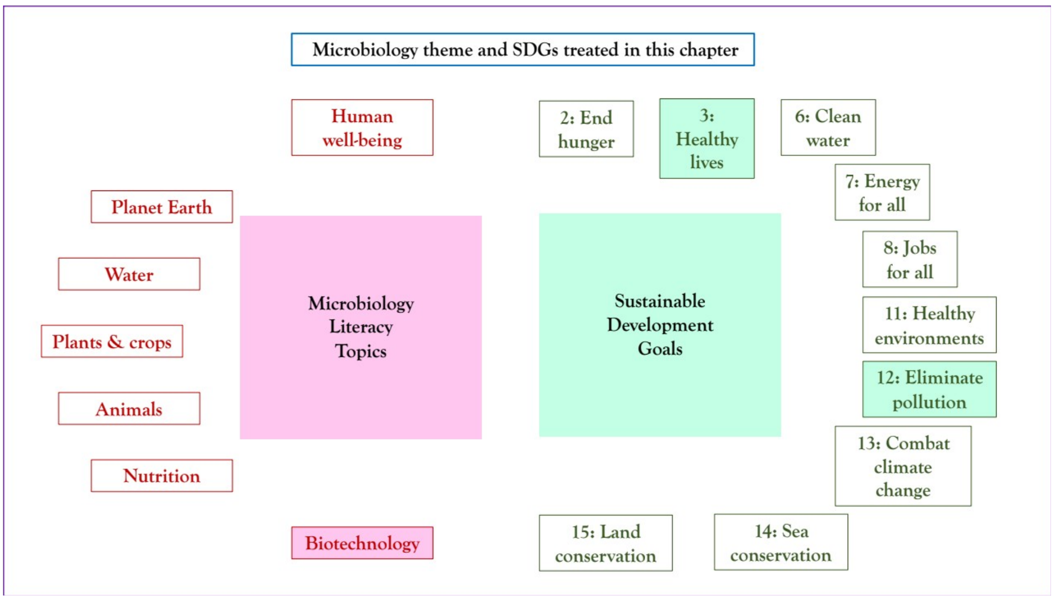
Storyline

Cells are surrounded by a membrane that separates the inside (cytoplasm) from the environment. The cell membrane is largely made of lipids, but there are also a number of proteins inserted into it that perform a variety of functions for the cell. These proteins naturally find their way from the cell cytoplasm to the membrane, where they decorate the surface of the microbe. It is possible to fuse a passenger protein of your choice to one of these membrane proteins, so that the passenger protein will extend out into the environment. This is called microbial surface display, because the protein is on *display* on the *surface* of the microbe. It is almost like part of the cell has been turned 'inside-out'. Microbial surface-display has many uses in biotechnology including in the processes for making protein therapeutics, the improvement of enzymes, and the development of diagnostic tests to identify diseases and pollutants. The reason surface display is useful is because the activity of the surface-displayed protein can be more easily assessed than if the protein is inside the cell. Therefore, if you have a population of cells that each have a different variant of the protein, you can find the best one much more quickly if those proteins are displayed on the cell. Surface display is a useful research tool for accelerating biotechnology projects and has positive effects on society because it allows the development of bio-based solutions for health and the environment and therefore contributes to meeting several of the sustainable development goals.

The Microbiology and Societal Context

The microbiology: Surface-display enables biotechnology; the projects are largely related to the production of medicines (human well-being), enzymes (planet earth via pollution reduction), and as sensors (human well-being because of disease diagnosis and planet earth because of pollution detection). *Sustainable development goals:* Healthy lives (production of new medicines and vaccines, disease diagnosis); Eliminate pollution (tests to detect pollution in water, soil, and air; identify enzymes to replace chemical synthesis).



Microbial Surface Display: The Microbiology

1. ***Cells are surrounded by a membrane composed of a mixture of lipids and proteins***. Cells are surrounded by a membrane that divides the inside of the cell from the surrounding environment. The cell membrane is made up of a double layer of lipids that keeps the inside of the cell from leaking out. But, in order to be able to bring food into the cell and expel waste, there are also some proteins in the membrane to perform specific functions. Because of the proteins, if we were to be able to zoom down and look at a cell, what we would find that it does not have a smooth surface like a beach ball, but instead a rough or hairy one like a tennis ball. This is because some of the proteins stick out away from the cell into the environment (Figure 2).

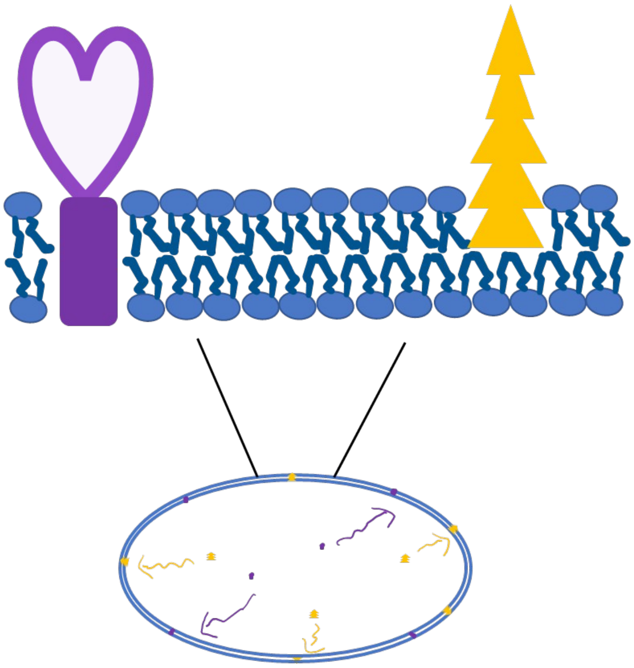


Figure 2. A cartoon of a cell membrane (blue) with proteins embedded in it (orange, purple). The proteins are made in the cytoplasm and travel to the cell membrane (arrows).

2. ***In biotechnology, it is possible to put 'passengers' onto membrane proteins***. If you are

interested in studying a protein, it is possible to glue it onto one of the membrane proteins as a 'passenger'. Then, when the membrane protein travels to the membrane, your protein of interest will also be brought along. The reason you might be interested in doing this is because it is easier to study your protein when it is on the outside of the cell compared to when it is inside. There are far fewer proteins there and you do not need to break open the cells to do your study. Hence, fusing your protein to a membrane protein as a passenger, allows you to turn the cells 'inside-out', at least partially. Scientists call this process surface display because your protein of interest is on display on the cell surface. The main benefit of surface display is that it speeds up the process of studying proteins and can allow you to find the best protein for your biotechnology project among a large number of possibilities (sometimes called a library).

In some cases, having a protein on the outside of the cell can also be convenient for doing chemical reactions, or detecting the presence of a molecule in the environment, because then the chemicals do not need to cross the cell membrane.

3. ***Using surface display for medical applications***. One of the applications of surface display is to find new protein therapeutics. Protein therapeutics are proteins that are used to treat diseases. A classical example of a protein therapeutic is insulin, which is used to treat diabetes by daily injections. There are also protein therapeutics for cancer, arthritis, and other long-term conditions. They can also be used to treat some infections. One type of protein therapeutic is called an antibody. Antibodies are naturally occurring proteins that are made by your immune system to fight diseases. Antibodies work by binding to a target and helping the body to eliminate it. Antibodies can also be made outside the body using biotechnology and injected into patients to help them fight a disease. Initially this was done by injecting animals, such as rabbits, with the target and then harvesting the antibodies generated by their immune system. Once the antibody is identified, it can be mass produced using biotechnology methods. In modern medicine, animal-derived antibodies are no longer used as therapeutics because of the potential for the immune system to recognize them as foreign substances and develop an undesired response against the therapeutic. Because of this, they have been largely supplanted by human or humanized antibodies. However, animal-derived antibodies still find wide application in diagnostics (see below).

Instead of using animals, surface display can be used to find antibodies that bind to a target, eliminating the need for animal experimentation. A library of antibodies, each of them slightly different, is made and each is displayed on the surface of a different cell. The antibodies that bind to the target the best can be identified by capturing the cells that display them with the target protein. This process is very quick and can sort through a large number of different antibodies (see Easter Egg Hunt, below). The fact that it is quick means that new treatments can reach patients more quickly. For example, in the SARS-Cov-2 pandemic, a form of surface display was used to identify antibodies against the virus in a matter of a few months. The speed also helps lower the costs of finding a new therapeutic because less time and effort is needed. This help keeps the cost of the medicines lower so that more patients can receive them. Therefore, overall, there are three benefits to using surface display to find new protein therapeutics: 1) speed, 2) cost, and 3) eliminating the need to use animals for experiments.

Another medical application of surface display is to create vaccines. Vaccines are medicines that are used to create an immune response that can prevent infections. Here, a passenger protein is displayed on an otherwise harmless cell to specifically mimic a disease-causing agent. The patient's immune system recognizes the protein and develops an immune response so that if the patient later encounters the actual disease-causing agent, they are already protected from getting the disease. A vaccine made by surface display is safe, because although it looks like the disease-causing agent from the outside, it is based on a harmless cell on the inside.

4. **Using surface display to develop biological synthesis routes for chemicals and consumer products**. Another application of surface display is in biocatalysis. Biocatalysis is the use of enzymes or whole cells to do chemical reactions (see…….). Biocatalysis is often a more environmentally friendly way to produce chemicals and materials than traditional chemistry because it can take place at low temperatures and in water, which avoids the need for

environment-polluting organic solvents. Enzymes are very specific and create fewer undesired side products, so overall enzyme processes produce less waste products than many chemical processes. Surface display can be used in biocatalysis in two ways. First, much like the identification of antibodies discussed above, surface display of enzyme libraries can be used to identify enzymes with improved characteristics (faster rates, higher stability, or a combination of these). Second, cells with enzymes displayed on their surface can be used directly in biochemical reactions – the cell acts as the enzyme - which are 'greener' than purely chemical methods. This is beneficial because it means that, in order to produce the enzyme, you merely need to grow a batch of cells—you do not need any further steps to purify the enzyme before using it. Moreover, because the enzyme is on the surface, the reactant does not need to cross the cell membrane to be converted to the product. Finally, at the end of the biochemical reaction, you can remove the enzyme more easily if it is attached to cells because the cells are heavy enough to sediment out of the reaction, compared to a free enzyme, which is too small and stays in solution.

5. **Applications of surface display to make diagnostic tests for the detection of disease or pollutants**. A recent application of surface display is to create tests that can detect proteins or chemicals (also called analytes) to help diagnose diseases or detect environmental contamination. Here, the protein displayed on the cell surface can bind to the analyte. Because each cell has many of the proteins on its surface and the analyte can be bound by more than one protein, the cells clump together when the analyte is present at high enough concentrations. The cell clumping is visible by eye and therefore can be an easy to use indicator of the presence of the analyte. Such diagnostic tests are inexpensive to manufacture because you only need to grow a batch of cells (much like in the biocatalysis example above).

6. **If you can do this with any type of cell, why are microbes useful?** Since all cells have a membrane, surface display could, in theory, be based on any organism. However, there are some advantages of choosing microbes for surface display. As single-celled organisms with relatively few requirements for growth, they are a low-cost, easy-to-use experimental platform. In addition, the genetic modification techniques necessary to glue the passenger protein to the membrane protein are easier to accomplish in microbes compared to more complex cell hosts. Finally, for applications in vaccines, they are good carriers of target proteins because they are the right size and shape to mimic disease-causing agents.

Relevance for Sustainable Development Goals and Grand Challenges

Surface display is an experimental method that speeds up the development of new biotechnologies. Therefore, it has broad impacts on development goals where biotechnology can be used to solve grand challenges. Using surface display will allow us to reach solutions to some of the grand challenges more quickly than using conventional genetic engineering/ selection methods. Some examples include:

- **Goal 3. Ensure healthy lives and promote well-being for all ages** (*end preventable deaths of newborns and children under the age of 5; end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases; reduce by one third premature mortality from non-communicable diseases; substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil* *pollution and contamination; support the research and development of vaccines and medicines for the communicable and non-communicable diseases*). Surface display can be used to accelerate the development of new protein medicines for a variety of communicable and non-communicable diseases, which both increases the variety of therapies available and decreases the costs of drug discovery. Surface display is also a useful, cost-effective technology for the production of vaccines for diseases, particularly for those that infect children and newborns. Finally, cells surface displaying proteins that can bind to targets can be used as the basis of a rapid testing platform that can be used to identify pollutants in the air, water, or soil and help direct remediation efforts.

- **Goal 12. Ensure sustainable consumption and production patterns** *(sustainable consumption and production; environmentally sound management of chemicals and all wastes throughout their life cycle; significantly reduce their release to air, water and soil).* Surface display enables the discovery of enzymes with faster reaction rates and enhanced stability, which contributes to the identification of biosynthetic routes to chemicals, materials, and consumer products as a replacement for petrochemical synthesis. This is expected to contribute to sustainable manufacturing and reduced pollution of air, water, and soil.

Potential Implications for Decisions

1. ***Individual***
   a. Supporting businesses that sell products that are made without animal experimentation.
   b. Supporting businesses that sell products made through environmentally friendly routes.

2. ***Community policies***
   a. Supporting large-scale testing of the environment, water, soil, or populations.

3. ***National policies***
   a. Limiting how much health services will pay for drugs or altering accessibility based on cost.
   b. Limiting greenhouse gas and other emissions by industries or putting in place taxes on said emissions.

Pupil participation

1. ***Class discussion***
   a. What does the surface of a microbe look like?
   b. How do we know, i.e. what kinds of experiments do scientists use to find this out?
   c. All cells have things on their surface, why are the benefits of using microbes for surface-display over other types of cells?
   d. What are some of the uses for 'inside-out' microbes?

2. ***Pupil stakeholder awareness***

a. Protein medicines are very expensive to make. Who pays for them? How can we ensure that everyone can have equal access to them?
b. Enzymes can substitute traditional chemical synthesis, often leading to less pollution and reducing the impact on the environment. An example is the dye in your jeans, which can be produced using many enzymes in a row. However, doing this is more expensive. How much more would you be willing to pay for something made by enzymes?

3. *Exercises*

a. Microbial surface-display can be used to create tests for infection or pollution that are rapid and easy to use. If you could make such a test, what would you want to detect? How would you use it, i.e. what would you do with the information?
b. Design/draw a new coat for your microbe. (Comparing drawings across the class gives a sense of the diversity of display libraries).
c. What are the pros and cons of the use of animals in research?

4. *Class experiments*

a. Easter Egg Hunt: To illustrate how putting a protein on the outside of the cell can be useful for sorting through a large number of variations quickly. Requires Easter eggs (or similar) and small objects like a marble, plastic shape, with different colours or shapes etc (the more the better). Put one object inside each egg and time how long it takes the students to find a specific one. Then ask them to imagine how much easier the task would be if the object was taped to the outside of the egg.

The Evidence Base, Further Reading and Teaching Aids

1. http://www.biology4kids.com/files/cell_membrane.html
2. http://www.biology4kids.com/files/cell_membprot.html
3. http://autodisplay-biotech.com/html/cell_surface_display.html
4. https://en.wikipedia.org/wiki/Bacterial_display
5. https://www.youtube.com/watch?v=GwQ51EMo62k (Includes too many molecular details for students and is fairly advanced, but there are not too many choices for such videos. Some useful visuals for teachers and those with biology training.)
6. Watson H. (2015). Biological membranes. *Essays Biochem*. **59**: 43–69. (open access source with more details on membrane structure, function, and methods for analysing membranes).
7. Jahns AC and Rehms BHA. (2012). Relevant uses of surface proteins – display on self-organized biological structures. *Microb. Biotechnol*. **5**: 188–202. (Open access, broad review that includes examples)
8. https://www.understandinganimalresearch.org.uk/animals/three-rs/ ; Singh J. (2012). *J Pharmacol Pharmacother*. **3**: 87–89 (Ethics of using animals in research and aims at reduction)
9. https://www.economist.com/business/2014/12/30/going-large (Costs of protein therapeutics and the challenges this brings for access to medicines)
10. Daugherty PS. (2007). Protein engineering with bacterial display. *Curr Opin Structural Biol.* **17:** 474-480. (Review of selection of antibodies by surface display)

11. Jegannathan KR and Neilsen PH. (2013). Environmental assessment of enzyme use in industrial production – a literature review. *Cleaner Production*. **42**:228-240. (Open access, discussion of how enzymes as environmentally friendly alternatives).; https://en.engormix.com/poultry-industry/articles/the-use-enzymes-profitable-t44648.htm (a specific example of enzymes in animal feed).
12. Shüürmann J, Quehl P, Festel G, and Jose J. (2014). Bacterial whole-cell biocatalysts by surface display of enzymes: toward industrial application. *Appl. Microbiol. Biotechnol.* **98**: 8031–8046. (Open access, review of surface display for biocatalysis).
13. Kylilis N, Riangrungroj P, Lai HE, Salema V, Fernandez LA, Stan GB, Freemont P, and Polizzi KM. (2019). Whole-Cell Biosensor with Tunable Limit of Detection Enables Low-Cost Agglutination Assays for Medical Diagnostic Applications. *ACS Sensors.* **2**: 370–378. (Open access example of sensing using surface display on bacteria)

Glossary

Analyte- A substance whose concentration is being measured in an analytical or diagnostic assay

Antibody- A naturally occurring protein in the immune system that recognizes and binds to foreign substances. The recognition event leads to downstream activities in the immune system that help eliminate the foreign substance

Biocatalysis- The use of whole-cells or enzymes to convert a reactant to a product

Cell membrane- A biological membrane, composed of lipids, that separates the inside of the cell from the environment

Cytoplasm- The inside of a cell

Enzyme- A protein that can accelerate a chemical reaction

Humanized Antibody- An antibody from a non-human species that has been changed to make it more human-like

Library- A large collection of biomolecules that differ slightly in sequence

Lipid- Fats or oils that are insoluble in water. In a cell, these are one of the primary components of the cell membrane

Organism- A living entity such as a cell, animal, or plant

Protein therapeutic- Proteins that are given to treat diseases

SARS-CoV-2 pandemic- An outbreak of viral disease that began in late autumn of 2019, resulting in global spread throughout 2019 and 2020

Vaccine- A substance given to a patient to simulate disease and promote an immune response that protects them when the real disease agent is encountered

Variant- Something that differs slightly from another thing of the same type. In biotechnology it is usually applied to proteins or nucleic acids and is used to describe members of a library.