Bringing "Life" Back to Life Science

Using Yeast to Enhance A Cell Unit

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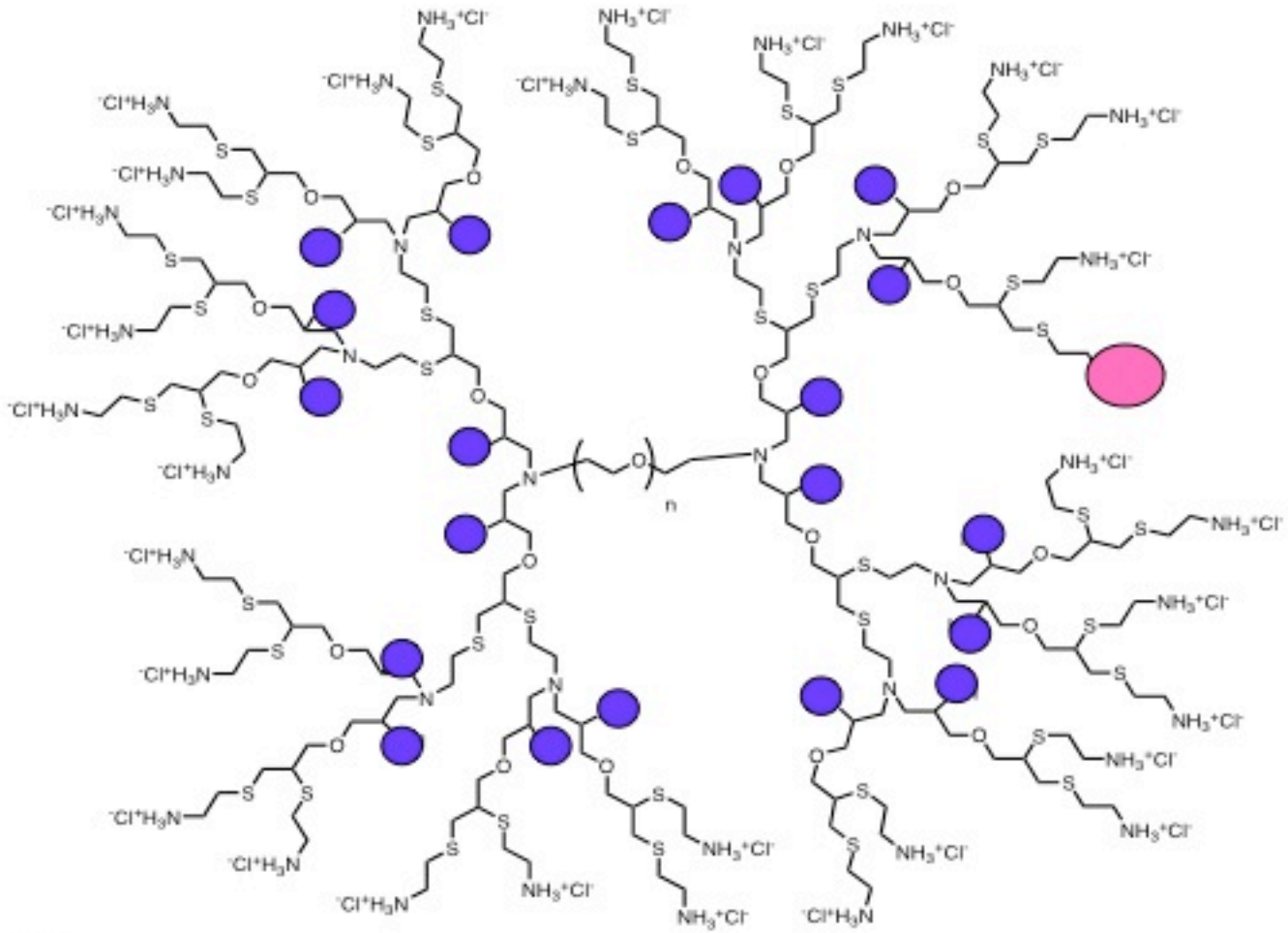
RET II

RET I Research

Developing Dendritic Drug Carriers

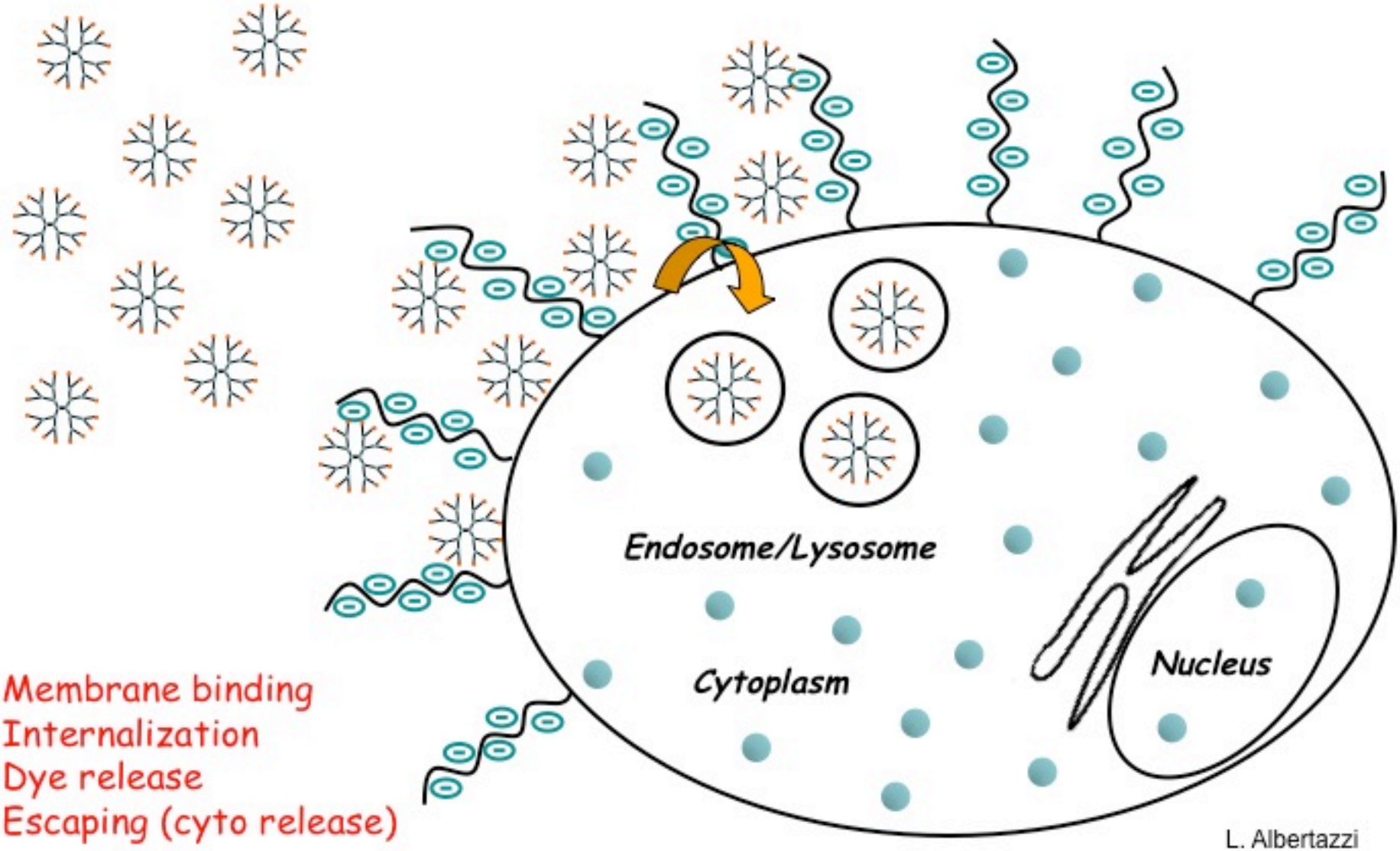
Mentor: Roey Amir

RET I Research



JW-6

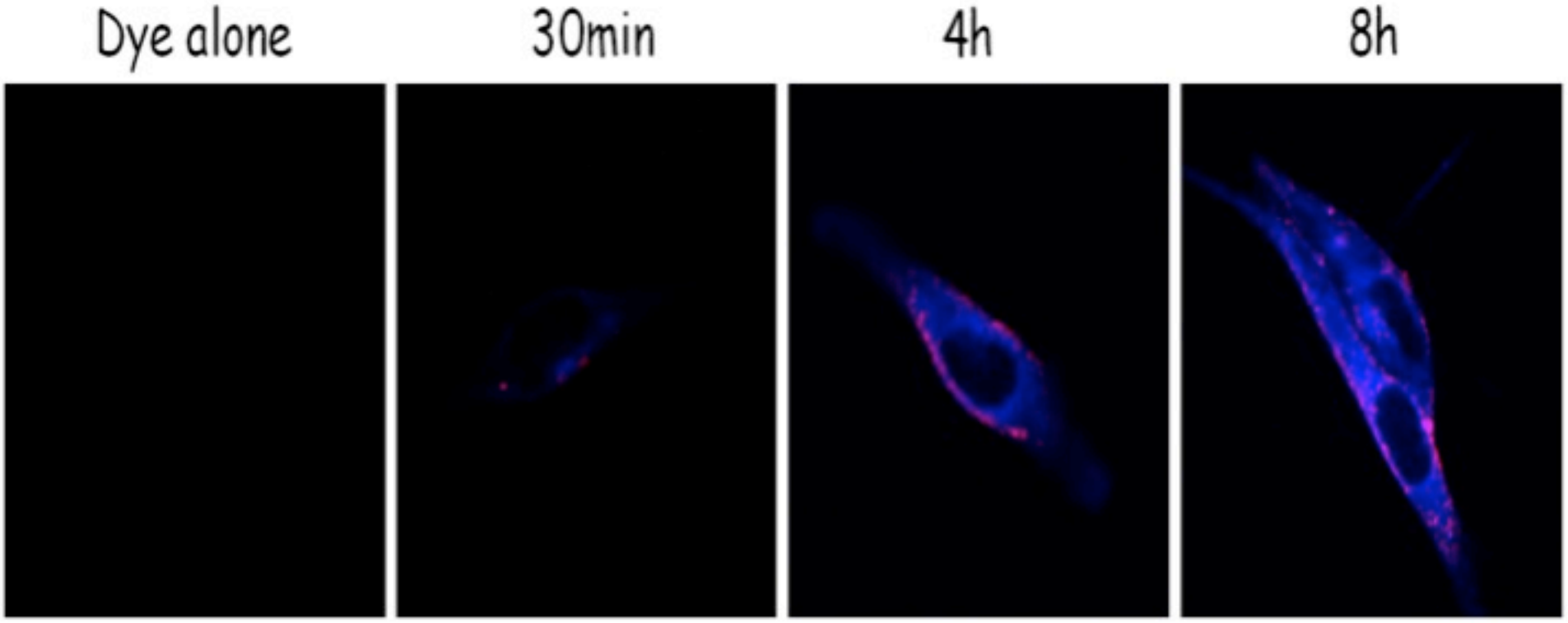
A (very) simple picture



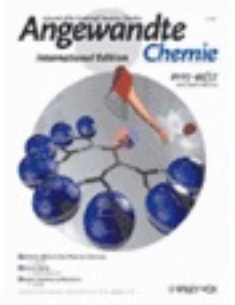
- Membrane binding
- Internalization
- Dye release
- Escaping (cyto release)

L. Albertazzi

Results: Internalization of both dyes attached to dendrimer.



Research published in ***Angewandte Chemie.***



Angewandte Chemie

Delivery Platforms

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Multifunctional Trackable Dendritic Scaffolds and Delivery Agents**

Roey J. Amir, Lorenzo Albertazzi, Jenny Willis, Anzar Khan, Taegon Kang, and Craig J. Hawker**

Dendrimers and other three-dimensional molecular assemblies are attractive scaffolds for biological delivery agents and diagnostic probes[1,2] due to their globular shape, modular structure, monodispersity, and plurality of functional end groups.[3] To address this potential, a number of strategies and related dendritic architectures have been developed for delivery of bioactive molecules to desired cells or tissue,[4] with encapsulation[5] and covalent attachment to the dendritic chain ends being two major approaches.[6] While the encapsulation of drugs or dyes within the inner cavities of the dendrimer is promising,[5] in most cases only a limited number of guest molecules can be encapsulated even with dendrimers of high generations.[4d] Moreover, the noncovalent nature of the encapsulation makes it a challenge to control the stability of the loaded carrier and subsequent release of the payload.[4e] An alternative strategy exploits the large number of dendritic chain ends to carry the cargo molecules.[6] However, loading of large amounts of hydrophobic drugs or dyes can alter the dendrimer surface properties and decrease its solubility and bio-compatibility.[7] Partial functionalization[8] alleviates this issue but results in random chain-end modification leading to a dispersity in loading, variable bio-performance, and in many cases only low degrees of surface functionalization can be achieved without significantly changing the surface properties.[9]

molecules to the interior of the dendrimer.[10] This strategy overcomes the challenges associated with surface functionalization, allowing high and reproducible loading without significantly altering the surface properties of the dendritic scaffold. To illustrate the power of this novel strategy, we report an accelerated synthesis of orthogonal surface and internally functionalized dendrimers[11] and their application as multifunctional dendritic scaffolds (Figure 1). As model

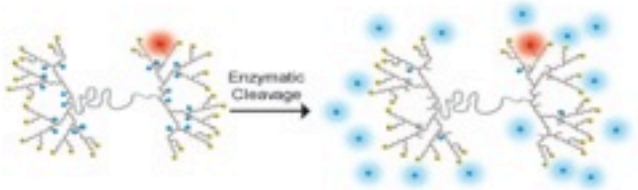


Figure 1. Modular design of dendritic scaffolds with protonated amino groups at the chain ends for cellular uptake (yellow). Upon enzymatic cleavage, the covalently attached, internal "blue" dyes are released while the noncleavable "red" dye allows for monitoring of the dendrimer itself.

delivery and diagnostic units, two different dyes were conjugated to the dendrimer: multiple coumarin units (blue

Current Classroom Observations:

- "Why do we need to learn this?"- more relevance
- "Do we get to do this today?"-more hands-on lab activities incorporated into the curriculum.
- Incorporate more of the scientific method into the curriculum

Connection to RET I

- Since our goal was to get a molecule into cells…
  - develop a unit around cells.
- A possible future application of dendrimers is as a drug carrier to target cancer cells…
  - incorporate a unit on skin cancer and introduce research.

Innovations:

- Collaboration on Lab Report Template
- Cohesive theme-prior knowledge for students to pull from
  - 2-quick labs
  - Investigative lab
  - 2 student inquiry labs

Cell membrane-Endocytosis
"A Feast on Yeast"

A Feast on Yeast!

Introduction:
Look through the transparent cell membrane of a one-celled organism, *Paramecium*, as it ingests yeast cells. You'll be amazed at what you see!

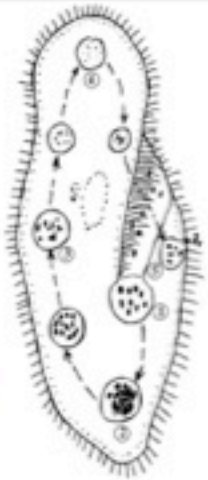
Background:

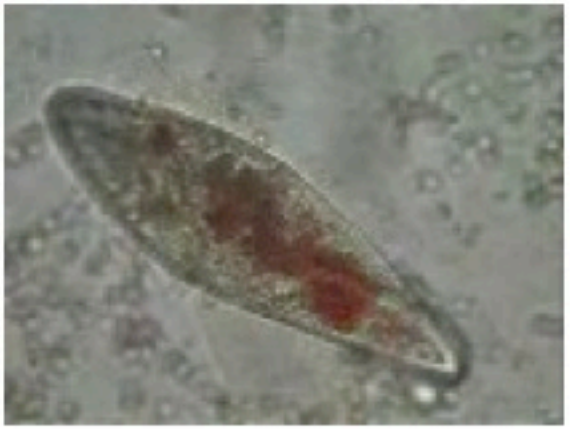
Endocytosis {Endo (within) cytosis (cell)} is a process in which a substance gains entry into a cell without passing through the cell membrane. Endocytosis is when a molecule causes the cell membrane to bulge inward, forming a vesicle. **Phagocytosis** is the type of endocytosis where an entire cell is engulfed.
Formation of food vacuoles is an example of endocytosis. Food substances such as bacteria and yeast are moved down the oral groove (gullet) and captured by a infolding of the plasma membrane in the formation of food vacuoles.
Paramecia are ciliated, one-cell organisms. They can be slowed with the use of methyl cellulose solution to allow careful observation of internal cellular activities. Stained yeast cells will enter a *Paramecium* through its oral groove. The stained cells can then be viewed as they circulate throughout the cell. The red stained yeast cells (inside the formed food vacuole) will likely be seen turning blue in color as pH changes occur during digestion. Congo red is red at pH 5 and is various shades of blue at lower pHs. An idealized path of the stained food vacuole might look like those in Figure 1.

Materials:

Paramecium culture	Slide coverslips
Stained yeast culture	Methyl cellulose solution, 3%
Microscope slides	Microscope
Colored pencils	

Procedure:
1. Place a ring of methyl cellulose on a clean microscope slide.
2. Place a small drop of *Paramecia* culture in the center of the methyl cellulose ring.
3. Add a small drop of stained yeast suspension to the *Paramecia* drop.
4. Cover with a clean coverslip and immediately observe with a microscope.
5. Locate a *Paramecium* and observe what happens to the stained yeast cells. The *Paramecia* will ingest some of the yeast cells very quickly, perhaps within 10 seconds of adding the yeast.
6. Study the food vacuole formed inside a *Paramecium* and watch it for at least 10 minutes. *Hint:* You will have to continuously focus up and down "through" the *Paramecium* and regulate the light carefully.
7. **Draw** a sketch of the *Paramecium* and show the path





Asexual Reproduction:
"Looking at Yeast…a Wee Little Beast."

Name _____ Date _____ Per. _____

Looking at Yeast…a Wee Little Beast

What is asexual reproduction?

Asexual reproduction is a form of reproduction, which requires only one parent. Unlike sexual reproduction, there is no exchange of genetic material or fertilization. Therefore, organisms that result from asexual reproduction will have the exact same DNA as their parent. Asexual reproduction is the simplest form of reproduction, since organisms are only making genetically identical copies of themselves. Asexual reproduction can be very advantageous to certain animals. Animals that remain in one particular place and are unable to look for mates would need to reproduce asexually. Another advantage of asexual reproduction is that numerous offspring can be produced without "costing" the parent a great amount of energy or time. Large colonies can form that can out-compete other organisms for nutrients and water. Large numbers of organisms mean that species may survive when conditions or the number of predators change. Environments that are stable and experience very little change are the best places for organisms that reproduce asexually. A disadvantage of this type of reproduction is the lack of genetic variation. All of the organisms are genetically identical and therefore share the same weaknesses. A negative mutation can make asexually produced organisms susceptible to disease and can destroy large numbers of offspring. Unfavorable conditions such as extreme temperatures can wipe out entire colonies. Some methods of asexual reproduction produce offspring that are close together and complete for food and space.

Yeast produce asexually by budding. In budding the cell wall pushes out, beginning the bud. The cell nucleus moves toward the bud and divides, with one nucleus moving into the bud and the other remaining in the parent cell. The bud grows, and eventually a cell wall grows between the parent cell and the bud. Finally, the bud breaks away and develops into a mature cell.

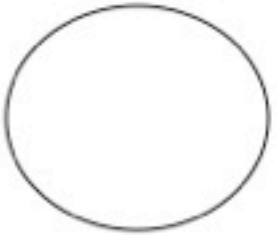
Objective: To look at common bread yeast *Saccharomyces cerevisiae* (sack-roe-MY-seas sair-o-VIS-e-eye) under the microscope. You will observe and sketch several yeast cells in different stages of budding.

Procedure:

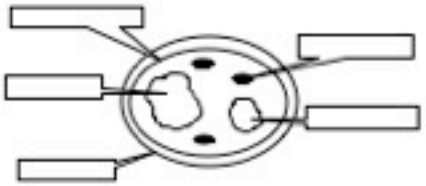
1. Get a slide and cover slip
2. Place 1 drop of the yeast solution onto the slide
3. Hold the cover slip at a 45° angle to the slide and lower the coverslip slowly to avoid any air bubbles.
4. Starting on low power, focus the microscope.
5. Switch to the 40x high power and focus the specimen, using **only** the fine focus knob.
6. Switch to the 100x high power and focus the specimen. You may need to adjust the diaphragm and the amount of light coming in.
7. Look for various yeast cells in different stages of budding. Accurately draw them in the space provided on the other side.
8. **Remember you will need to slowly move the slide around while continually adjusting the fine focus knob.**

Draw an **accurate picture with color** of yeast cells budding

Total Magnification_____



Simple yeast cell diagram:
Label the organelles



Questions:

1. Describe the appearance of yeast cells that are budding. Explain how this process works and how many cells are produced.

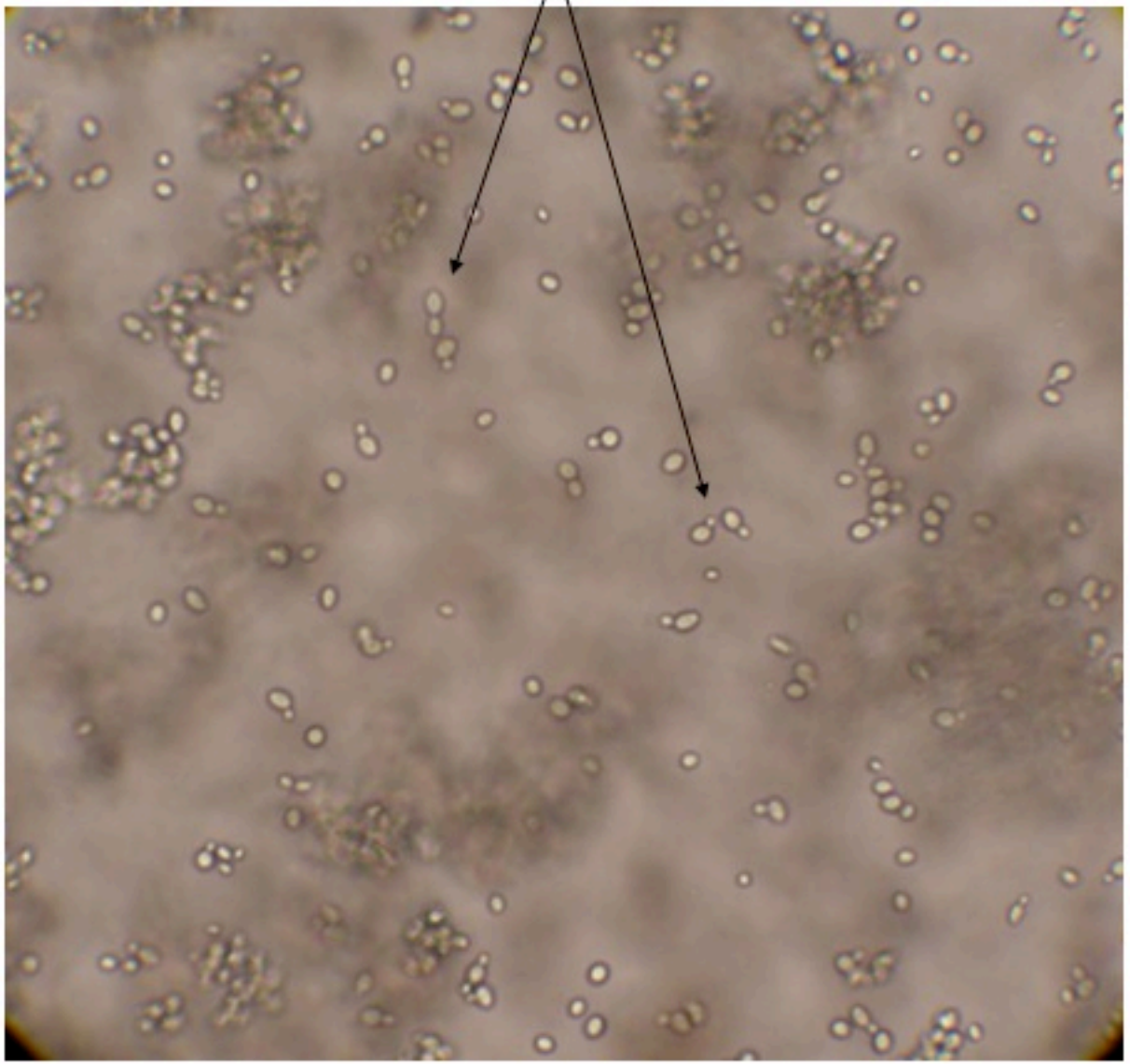
2. What is the difference between sexual and asexual reproduction?

3. What are three <u>advantages</u> of asexual reproduction?

4. What are three <u>disadvantages</u> of asexual reproduction?

5. Name some other organisms that can produce asexually?

Yeast Budding



Yeast Respiration/Fermentaion

"Yeast Respiration Lab"

- The purpose of this laboratory is to explore factors that might affect the rate of respiration in yeast.
- Students carry out an experiment to investigate and quantify the effect of sugar on yeast respiration.
- They will do this by observing yeast producing carbon dioxide gas.

Yeast can also go through cellular respiration without oxygen. This is also called **fermentation**. This is inefficient compared to respiration, but enables the yeast to survive and grow where no oxygen is available. In the absence of oxygen, fermentation partially breaks down the carbohydrate and a small amount of energy is captured in the form of ATP. The products are different depending upon the organism involved; in the case of yeast the products are ethanol and carbon dioxide.

Fermentation Equation:

$$C_6H_{12}O_6 \longrightarrow 2(CH_3CH_2OH) + 2CO_2 + \text{energy}$$
(alcohol)

We can respire in both ways too. Normally we use oxygen, but when we are running in a race, we may not get enough oxygen into our blood, so our muscles start to respire without oxygen. Unlike yeast we produce lactic acid, this causes the "burning" sensation and cramping in the muscles.

C. Why is measuring the amount of CO_2 an appropriate way to determine the amount of respiration in yeast? _____

_____

D. What waste product of yeast respiration is useful in making beer/wine? _____

E. What waste product of yeast respiration is useful in making bread? _____

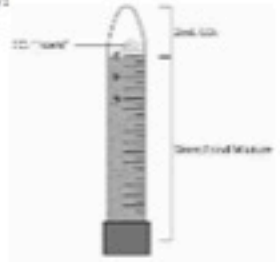
Part 2-

5. Pour yeast solution without sugar into tube labeled no sugar. Fill the tube all the way to the top, extending the fluid slightly above the top the tubes.
6. **Slowly** screw the cap on the tube, some may squirt out, this is O.K.
7. Turn the tube upside down and check to see that there is only a small bubble or bubbles. If there is a large bubble, you need to add more of the mixture to the tubes and try again.
8. Keep the tube upside down and place into one of the empty plastic cups. It is o.k. if some of the liquid leaks out!
9. Pour the sugar/yeast solution into the other tube labeled sugar.
10. Repeat steps 7-9.

***Now record the volume of carbon dioxide gas (CO_2) that is produced every 2 min. for 12 min. in the data table.**
***Remember the tube is upside down – make sure you read it correctly!**

Data Table:

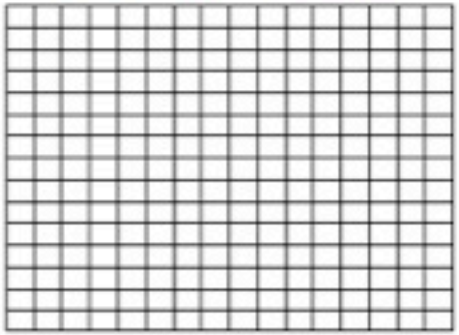
Time (minutes)	Total Volume of CO_2 Produced	
	Sugar	No Sugar
2		
4		
6		
8		
10		
12		



Graph:
Prepare a graph to summarize the data you recorded in the your data table.

- Label the Y-axis volume of CO_2 (ml); the X-axis time (min)
- Mark an appropriate scale
- Plot the data for yeast with sugar
- Plot the data for yeast with no sugar

The Effect of sugar on Respiration in Yeast



Conclusion Questions:

1. Was your hypothesis correct? Explain why or why not using data from the experiment.

   _____

   _____

2. Which set up was the control group? _____
3. Which set up was the experimental group? _____
4. What is the independent variable (what was changed) in this experiment? _____
5. What is the dependent variable (what was measured in this experiment? _____
6. Explain how the experiment may have produced data that was incorrect (sources of error).

   _____

   _____

7. What experiment would you test in the future that relates to the ideas in this lab?

   _____

   _____

CO_2 Production:

CO_2 Bubbles

Extension-Relevance: Use yeast solution to make bread!

- Pour yeast solution into cup.
- Add 4Tbsp. of flour and 1Tbsp. of warm water.

Inquiry Lab: Yeast Taste Test

- A follow up of previous activity-Does the **type of sugar** make a difference in CO_2 production?
- Students choose what to test and set up activity. Compare results to control from previous lab.

Lab Report Template:

Name: Date: Per:

Lab Report

Question:

Hypothesis:

Control:

Independent Variable:

Dependent Variable:

Procedure:

Materials:

Data/Results:

Conclusion:

A 1. Was your hypothesis correct? EXPLAIN why you accepted or rejected the hypothesis using data from the experiment.

2. What was the control? (If no control, why?)

3. Summarize your data.

B. 4. What were the independent and dependent variables?

5. Explain how the experiment may have produced data that was incorrect (sources of error).

C. 6. What experiment would you test in the future that relates to the ideas in this lab?

7. How does this experiment relate to what we are learning in class? BE SPECIFIC-use the correct vocabulary terms.

8. Describe how the information learned in this experiment relates to the real world.

Extensions/Discussion:

- This activity is also a great way to help students understand the importance of nutrition and metabolic processes within humans.
- Sugar is not the only thing we use for food. What would happen if we used other things?
- Why are there diet drinks? What is in diet drinks? What is in the sugar substitutes?

Cells/Genetics/Skin Cancer/UV light "SunScreen Your Genes"

- Students explore how effectively different sunscreens protect yeast cells from damage caused by exposure to ultraviolet (UV) radiation.
- Students may compare different SPFs, clothing, sunglasses
- The experiment utilizes a strain of yeast that lacks several DNA repair mechanisms
- Make students aware that simpler organisms can be used to help understand the processes in more complex organisms.

Introduction with PowerPoint on Skin Cancer

Sun Screen Your Genes

Student Handout

Instructions:

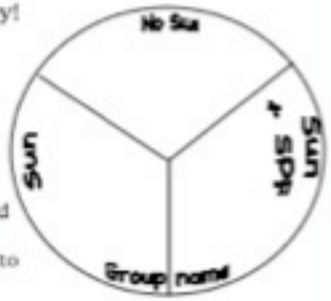
Does the SPF of your sunscreen really make a difference? Do some brands of sunscreen provide better protection than others? How effective are other forms of sun protection such as sunglasses or clothing?

You will have the opportunity to carry out an experiment that will help you answer these questions.

Why use yeast to study the effects of UV radiation?

Ordinary baker's yeast (*Saccharomyces cerevisiae)* contains genes for DNA repair that are very similar to human genes with the same function. Therefore we can use yeast as a model system to explore the effects of UV radiation on cells. *Like human cells, most yeast cells effectively repair DNA damage caused by UV radiation.* However, some yeast strains have mutations that prevent them from making certain types of DNA repairs. Because they cannot repair DNA damage, these cells usually die after exposure to UV radiation. This sensitivity enables us to observe how much DNA damage occurs when the cells are exposed to or protected from UV light.

Procedure: Follow all directions carefully!



1. Clean your hands and work area.
   - Wash your hands with soap and water.
   - Wipe your hands and your work area with alcohol and a paper towel.

2. Label your Petri dish using a waterproof marker.
   - Write your group name in small letters around the outside edge of the bottom of the dish.
   - Draw lines on the top and bottom of the dish to divide it into _____ parts.
   - Label one area "sun". Why?
   - Decide what sunscreens or other items you want to test in the other areas. You may want to have "no sun" in one area.
   - Label each area on both the top and the bottom of the dish.
   - Write in small letters around the edge.

3. Spread yeast cells on the agar in the Petri dish.
   - Swirl the container of UV-sensitive yeast.
   - Place 1 ml of yeast solution on the media.
   - Gently tilt and rotate the dish to spread the liquid.
   - If there are places the liquid does not cover, use the rounded end of a sterile toothpick to move the liquid over them.

4. Let the liquid soak into the agar.
   - Place the Petri dish in a dark place for 10-20 min. until the liquid disappears.

While you are waiting completely fill out your Lab Report: Question, Hypothesis, Control, variables, and Materials. You may put "see lab handout" under procedure.

5. Tape the 2 halves of the Petri dish together along the side.
   - Use small pieces of clear tape; do not place tape on the top of the Petri dish.
   - Make sure that the lines on the top and bottom halves of the Petri dish are aligned and that the label for each treatment is in the same area.

6. Spread sunscreen on the lid of the Petri dish.
   - Spread sunscreen in the places you marked; use an equal amount in each section and spread the sunscreen evenly.
   - Use the triple beam balance to measure out equal amounts of sunscreen.
   - In the area labeled "no sun", tape a square of dark paper over it.

7. Expose the Petri dish to the sun or to a UV light.
   - Make sure you have the dish pointed directly at the sun. With the smallest shadow possible.

8. Prepare to let the yeast grow.
   - Wipe the sunscreen off the lid of the Petri dish.
   - Place the Petri dish upside down in a dark place for 2 days.

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9. **Compare the amount of yeast that has grown in different areas of the Petri dish.**
10. **Fill out the rest of the Lab Report. Use the following Semi-qualitative method where growth is ranked on a scale of 0-4 for your data table.**
    - **0= no growth**
    - **1= little growth**
    - **2= some growth**
    - **3= high growth**
    - **4= maximum growth**
11. **Prepare to report your results to the rest of the class!**

Name: Date: Per:

Lab Report

Question:

Hypothesis:

Control:

Independent Variable:

Dependent Variable:

Procedure:

Materials:

Data / Results:

Conclusion:

A 1. Was your hypothesis correct? EXPLAIN why you accepted or rejected the hypothesis using data from the experiment.

2. What was the control? (If no control, why?)

3. Summarize your data.

B. 4. What were the independent and dependent variables?

5. Explain how the experiment may have produced data that was incorrect (sources of error).

C. 6. What experiment would you test in the future that relates to the ideas in this lab?

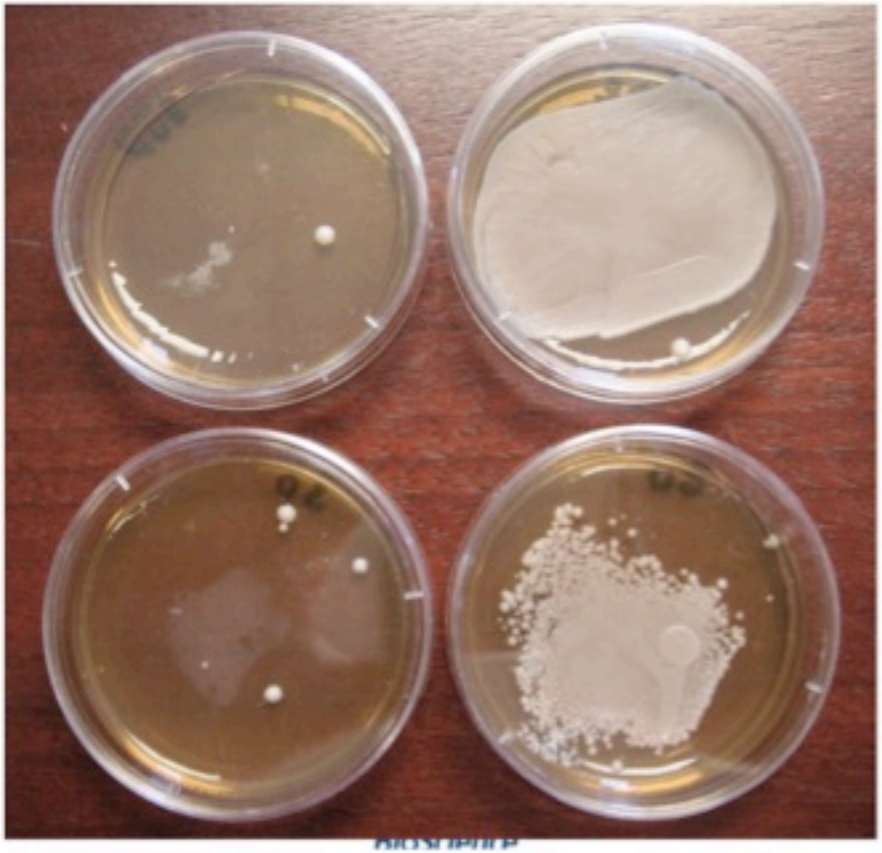
7. How does this experiment relate to what we are learning in class? BE SPECIFIC-use the correct vocabulary terms.

8. Describe how the information learned in this experiment relates to the real world.

Sample Results (a few days growth)

Full "Sun"

Covered/ No SUN



SPF 30

SPF 60

Discussion points:

- Ultraviolet light causes DNA damage. If unrepaired, this damage can *potentially* lead to mutations and skin cancer in humans.
- Unlike the yeast used in this experiment, DNA damage is often (but not always) repaired in humans.
- Sunscreens can be useful in reducing DNA damage caused by UV light.

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