

Experimental Self-Assembly

Yves Theriault, PhD, Program Manager, Education & Outreach (E&O), Qualcomm Institute
Executive Director, E&O, San Diego Nanotechnology Infrastructure (SDNI)
E&O Coordinator, UCSD Material Research Science & Engineering Center (MRSEC)
University of California San Diego

A. Chemical Self-Assembly

1. Background

1.1 What is Self-Assembly?

Self-assembly is a process where separate components spontaneously assemble and organize into ordered structures without external guidance. This phenomenon can be observed across various scales, from molecular to macroscopic levels, and is driven by specific, local interactions among the components themselves, such as electromagnetic forces, ionic and hydrophobic interactions, hydrogen bonding, and Van der Waals forces. Self-assembly plays a critical role in numerous natural processes, including the formation of cellular membranes, crystallization, and the biosynthesis and folding of biological macromolecules like proteins and DNA. It also has significant applications in nanotechnology, materials science, and biotechnology, enabling the creation of complex structures with precise functionalities from simple building blocks.

1.2 Self-Assembly in Medicine

Self-assembly in medicine represents a cutting-edge approach where this fundamental principle of nature is harnessed to develop innovative diagnostics, therapies, and drug delivery systems. This strategy leverages the ability of molecules and materials to organize themselves into functional structures without external intervention, offering several advantages for medical applications, including precision, scalability, and the potential for creating complex structures with minimal effort.

Self-assembling carriers, such as micelles, liposomes, and nanoparticles, can encapsulate drugs, protecting them from degradation and enhancing their delivery to specific sites within the body. This selective targeting can reduce side effects and improve the therapeutic efficacy of drugs. The first application of lipid nanoparticles, namely nanoliposomes, was in 1995 with the drug Doxil for cancer treatment where targeted delivery to tumor cells is critical. More recently, nanoliposome self-assembly was crucial in the development and formulation of the Pfizer COVID-19 vaccine in 2020.

1.3 Self-Assembly with Sodium Alginate and Calcium Chloride

Sodium alginate is a natural polysaccharide derived from brown seaweed that is widely used in the food industry, pharmaceuticals, and various industrial applications due to its unique properties as a thickener, emulsifier, stabilizer, and gelling agent.

In the food industry, sodium alginate is commonly used to improve the texture and viscosity of products like ice cream, jellies, dressings, and beverages. It's particularly valued for its ability to form gels in the

presence of calcium ions, a process called spherification, which is used in molecular gastronomy to create caviar-like beads and gel-coated spheres of liquid.

In pharmaceuticals, sodium alginate is used as an ingredient in antacid formulations, where it reacts with gastric acid to form a gel, providing a protective barrier for the lining of the stomach. It's also used in wound dressings due to its ability to absorb water and promote healing.

Beyond these applications, sodium alginate finds use in textile printing, papermaking, and as a binder in various industrial processes, showcasing its versatility and importance across different sectors.

When sodium alginate is added to a calcium chloride solution, a fascinating chemical reaction occurs, resulting in the formation of a gel. This process is a classic example of ionic gelation, leveraging the interaction between the negatively charged carboxylate groups in the alginate and the divalent calcium ions Ca^{+2} .

Here's what happens step by step:

Dispersion: Sodium alginate, a polymer, is dissolved in water, where its molecules are dispersed throughout the solution. In this state, the alginate's carboxylate groups (COO^-) are ionically bonded to sodium ions (Na^+).

Ion Exchange: When this solution is introduced into a calcium chloride (CaCl_2) solution, calcium ions (Ca^{+2}) are available in the environment. These calcium ions have a higher affinity for the carboxylate groups than the sodium ions, leading to an exchange. The calcium ions begin to replace the sodium ions attached to the alginate.

Cross-Linking: Calcium ions have the ability to bind with several alginate molecules simultaneously because they can form ionic bonds with the carboxylate groups on different alginate chains. This cross-linking action pulls the alginate molecules closer together, forming a three-dimensional network throughout the solution.

Gel Formation: The result of this cross-linking is the transformation of the liquid alginate solution into a gel. The gel's firmness and porosity can vary based on the concentration of alginate and calcium chloride, as well as the ratio of mannuronic to guluronic acid units in the alginate. Guluronic acid-rich alginates tend to form stronger, more brittle gels with calcium ions.

This reaction is the basis for the spherification technique in molecular gastronomy, where small amounts of flavored liquid containing sodium alginate are dropped into a calcium chloride solution, forming gel-coated spheres that burst with flavor when eaten. It's also utilized in biomedical applications for encapsulating cells or drugs, due to its biocompatibility and the mild conditions under which gelation occurs.

Analogy with the covid-19 nanoliposome self-assembly

The analogy between the gelation process of sodium alginate when added to a calcium chloride solution and the self-assembly of COVID-19 nanoliposome particles lies in the principle of interaction-driven assembly to achieve a desired structure and function. Both processes involve the assembly or transformation of components at the nanoscale or molecular level through interactions that are specific

and controlled. However, the substances involved, and the nature of the interactions differ significantly. Let's explore both processes to highlight the analogy:

Sodium Alginate Gelation

Components: Sodium alginate (polysaccharide) and calcium ions (Ca^{2+}).

Mechanism: The process involves ionic cross-linking. Sodium alginate, dissolved in water, interacts with calcium ions when mixed with a calcium chloride solution. The calcium ions serve as cross-linking agents that bind to the alginate chains, causing the polymer to gel.

Result: The formation of a gel network, where the physical properties of the gel (such as its strength and elasticity) can be controlled by the concentration of alginate and calcium ions, as well as the alginate's composition.

COVID-19 Nanoliposome Self-Assembly

Components: Lipids (including phospholipids and possibly cholesterol) and potentially other molecules designed to mimic aspects of the SARS-CoV-2 virus structure or to encapsulate therapeutic agents.

Mechanism: Self-assembly of nanoliposomes involves the spontaneous organization of lipids in an aqueous environment into bilayered vesicles (liposomes). This process is driven by the hydrophobic effect, where the hydrophobic (water-fearing) tails of the lipids seek to avoid water while the hydrophilic (water-loving) heads interact with the aqueous environment, resulting in a bilayer structure that can encapsulate substances.

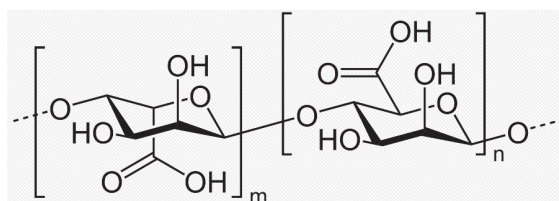
Result: The formation of nanoliposomes, which can be engineered to deliver drugs, vaccines, or other therapeutic agents to specific sites in the body. In the context of COVID-19, these nanoliposomes can be designed to mimic certain features of the virus to trigger an immune response or to deliver RNA vaccines or therapeutic agents directly to cells.

Analogy

The analogy lies in the self-organizing or interaction-driven transformation of components into a structured assembly for a specific purpose—gel formation in the case of sodium alginate and vesicle formation for nanoliposomes. Both processes utilize the intrinsic properties of the materials involved: ionic interactions for alginate and hydrophobic/hydrophilic interactions for lipids (it should be noticed that the binding of ionizable cationic lipids to the mRNA is ionic in nature) to achieve a controlled and functional outcome. However, the specific mechanisms (ionic cross-linking vs. hydrophobic effect-driven assembly) and purposes (gelation for various applications vs. targeted drug/vaccine delivery) highlight the versatility of interaction-driven assembly processes in both material science and nanotechnology.

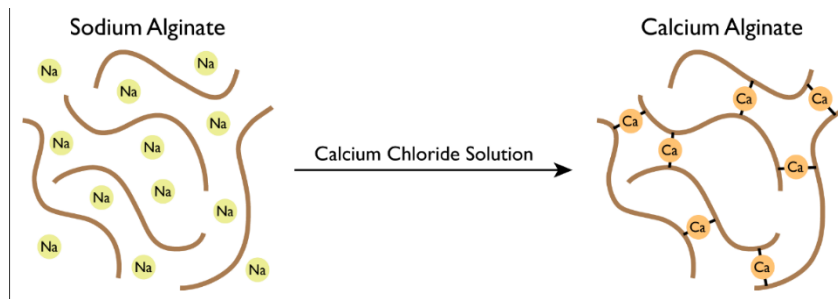
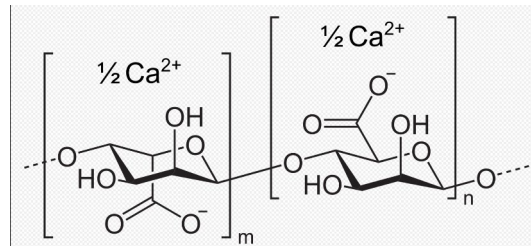
Chemical Structure of Alginic Acid

Source: https://en.wikipedia.org/wiki/Alginic_acid#/media/File:Algins%C3%A4ure.svg



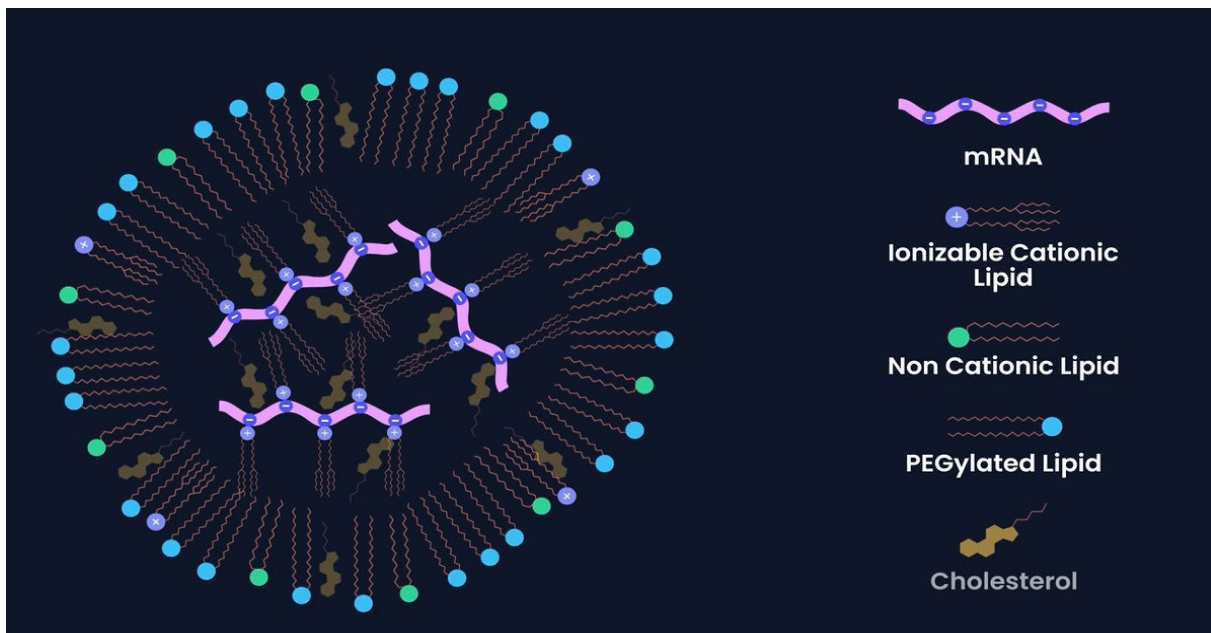
Chemical Structure and Molecular Model of Sodium Alginate and Calcium Alginate

Source: https://en.wikipedia.org/wiki/Calcium_alginate#/media/File:Calcium_alginate_skeletal.svg



<https://scienceandfooducla.files.wordpress.com/2013/06/alginategelation1.png>

Molecular model of liposome-embedded mRNA in Pfizer COVID-19 vaccine



Source: Theriault 2023 To see video, click [HERE](#)

2. Chemical Self-Assembly Laboratory

2.1 Materials

- 1 pack of Sodium Alginate (food grade)
- 1 pack of Calcium Chloride (food grade) (bulk)
- 1 Measuring cup or cylinder
- 1 8-12 oz cup
- 8-10 small cups (to contain 50 ml of sodium alginate solution/group of 4)
- 1 Blender
- 8-10 pipettes (1/group of 4)
- 8-10 small strainers (1/group of 4) or home-made net screen cones (1/group of 4)
- 5 oz of Food Colorant ($\frac{1}{2}$ oz /group of 4). Green color recommended.
- 1 7-inch diameter aluminum circular pan

2.2 Methods

2.2.1 Preparation of sodium alginate by teacher

Sodium Alginate solution. Suggested to prepare the sodium alginate solution the day before the experiment.

- Add one teaspoon of sodium alginate powder (food grade) with two cups of water (or 500 ml) in a blender.
- Blend until homogeneous solution is obtained (no powder should be deposited on the side or bottom of the blender).
- Transfer in a see-through 16 oz cup or in two 8 oz cups.
- Let sit for 30 minutes until no bubble is observed in the solution.
- Transfer 50 ml of the bulk solution into test tubes or small glasses. You will need 20 ml of solution for each team of 4 students.

- ***On the day of the experiment, provide each team of 4 students with 20 ml of sodium alginate solution, one 3-ml pipette, and one small strainer. In addition each group should receive 1 teaspoon of calcium chloride, a 7-inch diameter aluminum pan, 3 cups of water at room temperature, and $\frac{1}{2}$ oz of food colorant.***

2.2.2 Preparation of calcium chloride solution by students

The first step consists of preparing the calcium chloride solution by students on the day of the experiment.

Each group of 4 students perform the following:

- Put 3 cups of water in a 7-inch diameter circular aluminum pan.
- Add 1 teaspoon of calcium chloride (food grade).
- Use a spoon to stir solution until no solid particle of calcium chloride can be seen.
- Add $\frac{1}{2}$ oz of food colorant (green suggested). Stir until the solution is homogeneous.

2.2.3 Experimental Procedures

1. Put the small strainer in the center of the aluminum pan containing the colored calcium chloride solution.
2. Press on the tip of the pipette and insert on sodium alginate solution. Pipette sodium alginate and fill the pipette (the pipette may not fill entirely).
3. One drop at a time, drop the entire content of the pipette. Repeat 3-4 times.
4. Remove the strainer from the aluminum pan and set aside.

2.3 Results and Discussion

1. What do you observe in the strainer?
2. What color is it?
3. How do you explain the shapes that you see?
4. How do you explain the color that you see?
5. Does the experiment help you to understand self-assembly?
6. Provide your own definition of self-assembly.
7. Give 2 examples of real-world applications of self-assembly.

2.3.1 Answer Key

1. Spherical gel structures.
2. The spherical gel structures are green.
3. Positively charged calcium ions bind to several negatively charged alginate long chains forming the spherical beads, a process called spherification. Spherification is a self-assembly process.
4. The green colorant molecules are trapped in the beads as a result of spherification.
5. Answers will vary. The process observed is spontaneous and requires only to add sodium alginate to a solution of calcium chloride for spherification to occur.
6. Answers will vary. A definition of self-assembly is provided in the background.
7. Answers will vary. Examples are: Spherification observed in this lab activity. Formation of NaCl from Na^+ and Cl^- . Gold nanoparticle formation from self-assembly of gold atoms. Biding of ionizable cationic lipids to negatively charged phosphate groups of the mRNA and formation of liposomes in the COVID-19 vaccine formulations.

B. Magnetic Self Assembly

1. Background

1.1 What is Self-Assembly?

Self-assembly is a process where separate components spontaneously assemble and organize into ordered structures without external guidance. This phenomenon can be observed across various scales, from molecular to macroscopic levels, and is driven by specific, local interactions among the components themselves, such as electromagnetic forces, ionic and hydrophobic interactions, hydrogen bonding, and Van der Waals forces. Self-assembly plays a critical role in numerous natural processes, including the formation of cellular membranes, crystallization, and the folding of biological macromolecules like proteins and DNA. It also has significant applications in nanotechnology, materials science, and biotechnology, enabling the creation of complex structures with precise functionalities from simple building blocks.

2. Magnetic Self-Assembly Laboratory

2.1 Materials

The following describes the materials needed for 1 self-assembly kit. The experiment can be conducted using only one kit by the teacher as a demo with the entire class. Alternatively, several kits can be provided to teams of 2-4 students.

- 30 one-inch diameter flat circular pieces of foam with different colors on each face.
- 30 neodymium magnets 3 mm (wide) x 1 mm (thick)
- 1 tube of Super glue or Gorilla glue
- 1 rectangular plastic (better material) pan 9" x 13" or aluminum pan (magnets have a tendency to bind to the sides of the aluminum pans which introduces a bias; however the experiment can be conducted with small magnets where affinity for the pan is not too strong; aluminum pans are more affordable to scale up).
- 1 gallon of tap water
- Pairs of Vinyl or Nitrile Gloves (one pair for each person assembling the magnet/foam pieces). It may take more than one pair per person if glue comes in contact with gloves.

2.2 Procedures

Safety Warning: The glue is very strong, and injuries may occur if the glue comes in contact with the any part of the body. Special precautions should be taken to avoid contact with the eyes. **Safety glasses should be worn at all time while operating with the glue.**

2.2.1 Addition of magnets to circular foam pieces by teacher

- If several kits are to be provided, it is recommended that the teacher works with teaching assistants and/or 2-3 champion students. It is not recommended to ask all teams in a class of 25-40 students to glue the magnets to the foam pieces.
- First off, start by piling the magnets in one stick. All magnet will have the same polarity on the front and back side.
- With a permanent black marker, color the bottom part of your magnet stick and remember to always have that side on the right when taking magnets off the stick to glue them to foam pieces (one at a time).
- The foam pieces must have a different color on each side. Let's assume that we have yellow on one side and red on the other.
- Put a small quantity of glue in the middle of a foam piece. Glue all magnets on the yellow side of the foam pieces.
- Take a magnet of the stick by sliding it on the top. Pulling on it will not work well.
- Bring the magnet close to the glue at the center of the foam and let it fall on the glue/foam. Do not touch the glue!
- Bring the magnet stick underneath the foam around the center. Make sure that the black marked side is at the bottom. If the magnet is on the wrong side (we want all magnets to have the same polarization on the same side of the foam (same color), it will flip. If it is in the correct orientation, it will be still. Drive the stick around to adjust the position of the magnet to the center of the foam. It is very important that the magnets are centered correctly.
- Repeat for 30 foam pieces.
- Let dry in the aluminum pan for 24 hours.

2.2.2 Experimental self-assembly by students (we will use below the word foam in lieu of foam piece)

Part A. Have students follow these instructions:

1. Fill the aluminum pan to 2/3 capacity.
2. Put a foam with the red side up in the center of the pan (in the water).
3. Add one foam with yellow side up at 2-3 inches from the red foam.
4. Take note of what is happening. Draw what you see on a piece of paper.
5. Add another foam with the yellow side up at 2-3 inches of the self-assembled foams at an angle of 90 degree from the imaginary line linking the self-assembled foams.
6. Take note of what is happening. Draw what you see on a piece of paper.
7. Repeat step 5 and take note of what is happening. Draw what you see on a piece of paper.
8. Add an additional foam with yellow side up at any angle but at 2-3 inches from the red foam.
9. Notice what is happening. Draw what you see on a piece of paper.
10. Repeat 8, but this time, if necessary, push the yellow foam to the vicinity of the red foam to bind it with the red foam.
11. Take note of what is happening. Draw what you see on a piece of paper.
12. Repeat steps 2-11 in another area of the pan and make sure that the two self-assembled entities do not act on each other.

13. Gently bring the self-assembled entities to opposite sides of the pan.
14. Add one foam red side up in the middle of the pan.
15. Gently bring one of the first self-assembled entity in the vicinity (2 inches) of the red foam.
16. Note what happens. Draw what you see on a piece of paper.
17. Repeat with the other self-assembled entity. Bring 2 inches close to the red foam but from the opposite side compared to step 15.
18. Take note of what is happening. Draw what you see on a piece of paper.
19. Explore other self-assembly experiment on your own. Make sure to take note of the sequence of events. Draw what you see from start to end.

Part B. Have students follow these instructions:

1. Start with a pan filled with water at 2/3 capacity; no foam.
2. Add 30 foams with red side up one at a time putting them in the middle of the pan. Wait 3 second between the addition of two consecutive foams.
3. What do you observe during the process of adding the foams? What do you observe after adding all 30 foams? Take a picture of what you see.
4. Selectively turn foams to the yellow side one at a time in different areas of the pan. Note and draw what you observe.

Cleaning

Remove all foams from the water and deposit them on a paper towel to dry with yellow side up.

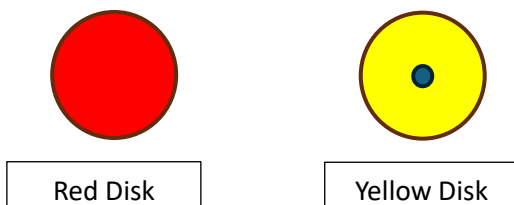
Leave the pan on your desk/table. Your teaching assistant or teacher will take care of it.

2.3 Results and Discussion

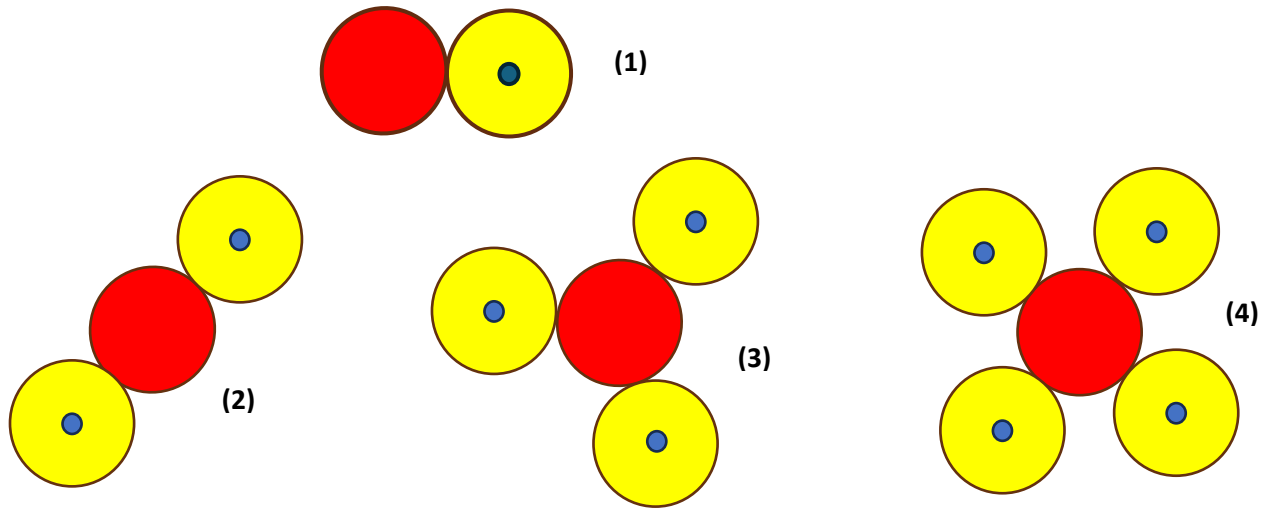
1. What are the 2 major forces acting on your magnetized foam disks?
2. Describe and explain what happened in Part A, steps 1-11 of this lab activity?
3. Describe and explain what happened in Part A, steps 12-15 of this lab activity?
4. Describe and explain what happened in Part A, step 17 of this lab activity?
5. How do you explain what happened in step 1-3 of Part B of this lab activity?
6. How do you explain what happened in step 4 of Part B of this lab activity?
7. Explain self-assembly in your own words.
8. Can the understanding of self-assembly be applied to the world of chemistry. i.e. atoms, molecules, ions? Explain in your own words.
9. Watch the video on the Pfizer's Covid-19 vaccine formulation and explain how self-assembly plays a role in the vaccine formulation.

2.3.1 Answer Key

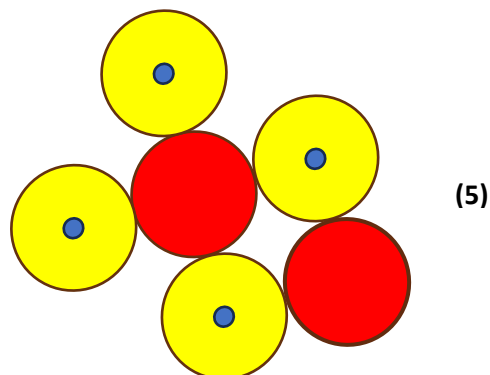
0. Here, we assume that the yellow side is the side attached to the magnets. We will refer to Red and Yellow disk in accordance with the side of the foam that faces up in the pan.



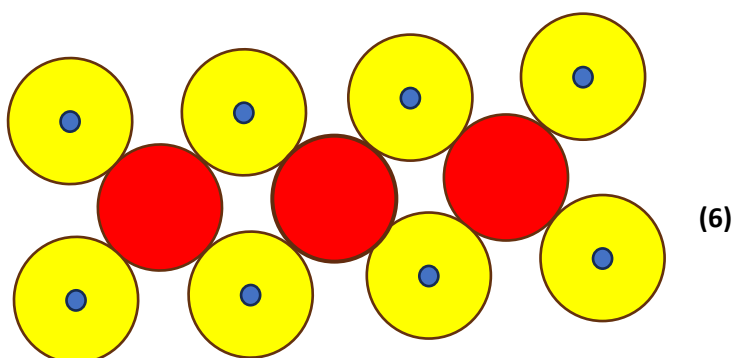
1. The forces are attractive and repulsive force. Disks of same color repulse each other and disks of different colors attract each other.
2. Answers may vary, but students should be able to identify the following patterns:



- When adding a yellow disk in the vicinity of a red disk, the yellow disk binds to the red disk.
 - When adding a second yellow disk, the latter binds to the red disk but also take a distance from the other yellow disk (already bound to the red) as yellows repulse each other. The final configuration should be as depicted in pattern (2) above (linear configuration).
 - When adding a third yellow disk, the latter binds to the red disk but also take a distance from the other yellow disk (already bound to the red) as yellows repulse each other. The final configuration should be as depicted in pattern (3) above (triangular configuration).
 - When adding a fourth yellow disk, the latter does not bind spontaneously to the red disk because the surrounding yellow disks exert a repulsive force preventing the binding. So, a force needs to be applied to the fourth yellow disk in order for it to bind to the central red disk. Again, once bound, the 4 yellow disks repulse each other leading to the configuration depicted on pattern (4) The final configuration should be as depicted in patterns (4) above (square configuration).
3. Answers may vary, but students should be able to identify patterns similar to the pattern (5)



4. Answers may vary, but students should be able to identify patterns similar to the pattern (6)



5. All disk having the same polarity. They all repulse each other and distance themselves to occupy the maximum space in the pan. The boundaries of the pan are unbreakable energy barriers, so the foam cannot move further, and this has an effect on the entire network due to the positions of those disks and the repulsive forces that they exert in their surroundings. Pictures using cell phones here would be appropriate in lieu of drawing the entire configuration.
6. Here several pictures can be taken in the course of flipping an increasing number of red disks. Students will observe that the same patterns observed in Part A are taking place, but sometime the configuration is distorted because one of the disk is positioned against the side of the pan, or is surrounded by other disks of varying forces. It is not possible to draw all possible configurations, but the concept of long-range interaction should be introduced here.
7. Answers will vary. The background section provides a definition of self-assembly.
8. Ionic bounding such as Na^+ binding to Cl^- to form NaCl is an example of self-assembly. The self-aggregation of gold atoms to form gold nanoparticles is another example of self-

assembly. Organic Chemistry can also be considered self-assembly: when put in the proper conditions, atoms of the reactants recombine to form products that have different structures and compositions compared to the reactants.

9. Self-assembly of the lipid molecules is responsible for formation of liposome and the embedment of the mRNA as well as its delivery to the cells.

Credits and Disclosure Statements

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