Gas Vesicle Nanoparticles

By: Willow Miranda

Gas vesicle nanoparticles are cylindrical or spindle-shaped organelles that are found in both Bacteria and Archaea.

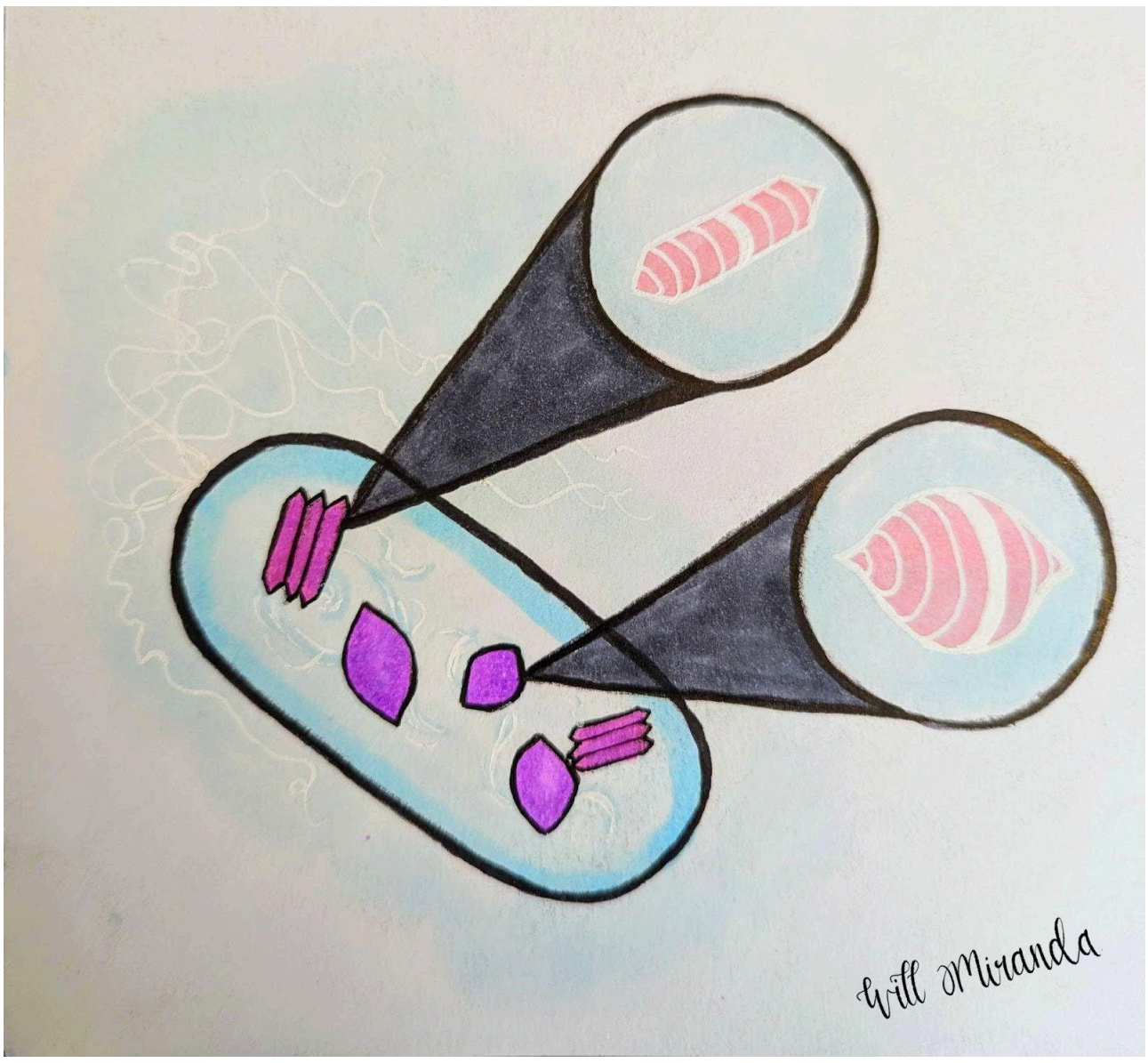


Fig. 1. Artist's rendition of cell containing GVNPs. Zoomed in depictions of cylindrical and spindle-shaped GVNPs. Based on electron micrographs from DasSarma & DasSarma, 2021. © 2024 Willow Miranda

Gas vesicle nanoparticles, GVNPs for short, are surrounded by a lipid-free single layer ribbed protein membrane, which is believed to be unique in nature. The membrane is gas-permeable but not liquid-permeable, keeping the internal environment free of the cytosol. It is also extremely stable, being so insoluble and resistant to denaturation that scientists have been unable to fully take them apart (DasSarma & DasDarma, 2021).

However, just because the membrane cannot be disassembled, it does not mean that we know nothing about the inside. GVNPs are not invulnerable. In 1895, German scientist Heinrich Klebahn found that under significant enough hydrostatic pressure GVNPs will collapse. This cave-in releases gas with the same composition as the atmosphere and caused the cyanobacteria he was working with to sink.

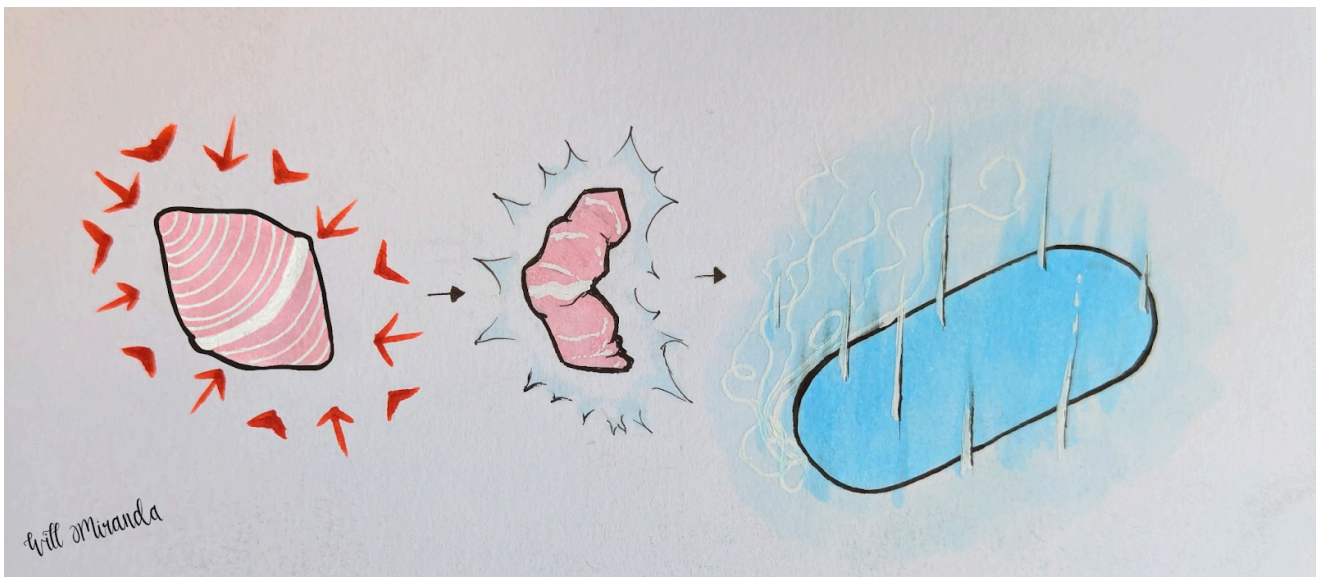


Fig. 2. Artist's rendition of a GVNP collapsing and microorganism sinking. Based on electron micrographs from [1]. © 2024 Willow Miranda

Heinrich's experiments proved that GVNPs are gas-filled and play a role in controlling the floatation of cells. Indeed, GVNPs are mainly found in aquatic organisms, and their purpose is to promote the vertical movement of cells in the water. This allows prokaryotes with GVNPs to float closer to the sunlight, evade predators, escape anaerobic conditions, find a warmer environment, or simply position themselves advantageously, based on their species. GVNPs are also refractive, which may protect organisms from harmful UV radiation. In addition to the floatation from GVNPs, these microorganisms often have increased mobility from flagella or similar structures, allowing them to swim.

For instance, planktonic cells without gas vesicles were observed to sink at a rate of two to four centimeters per day, while plankton with GVNPs floated, giving them greater access to sunlight for photosynthesis or phototrophy [1].

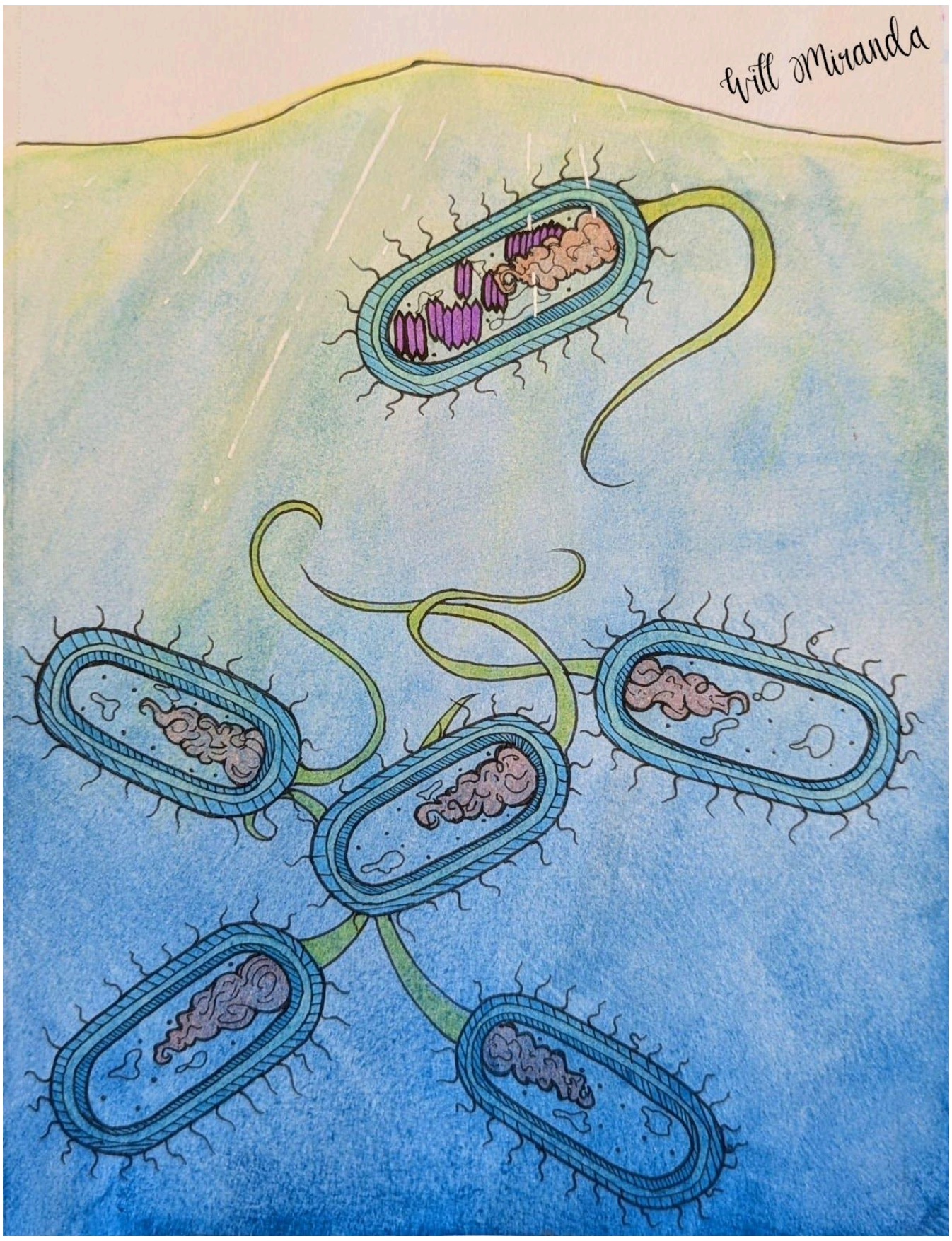


Fig. 3. Artist’s rendition of effect of GVNPs on flotation. Top organism contains GVNPs, letting it float to the area with more sunlight. Other five organisms lack GVNPs and sink. Partially based on electron micrographs from [1]. This image does not depict haloarchaea.

Whether an organism’s GVNPs are spindle-shaped, long cylinders, short cylinders, or some combination thereof seems to depend on the depth at which it lives. If the external hydrostatic pressure on the GVNPs becomes too much greater than the internal turgor pressure, it can collapse. Since hydrostatic pressure increases with depth, cells that inhabit deeper waters need gas vesicles that are more resistant to collapse. To this end, organisms like some cyanobacteria, which live deeper underwater, tend to have narrow cylindrical gas vesicles that are less collapsible. Meanwhile, some purple sulfur bacteria and halophilic archaea, which inhabit shallow, briny ponds, tend to have spindle-shaped vesicles. In between these two extremes are the organisms with wider cylinders. These shapes would collapse at greater depths, but they work well in shallow water [1].

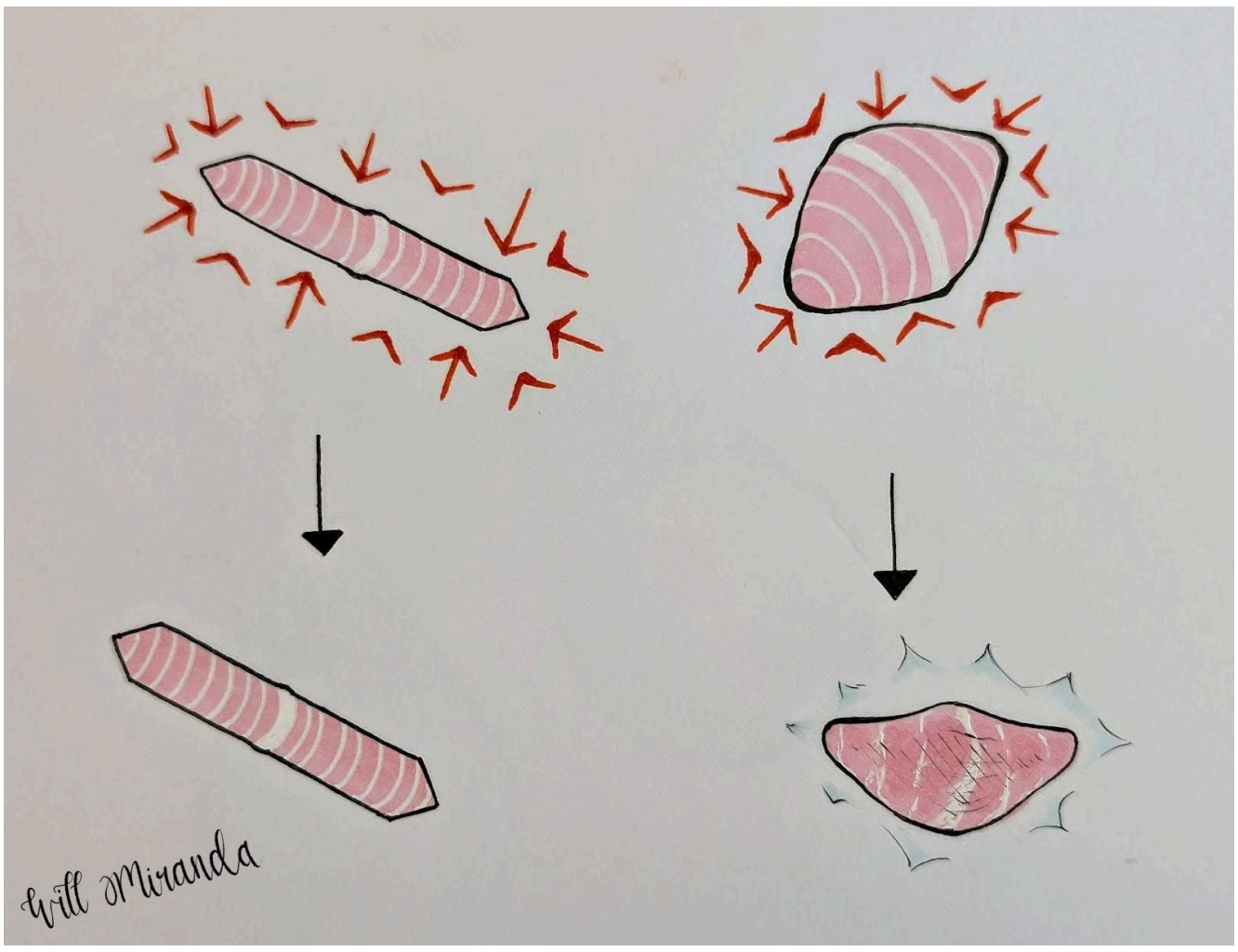


Fig. 4. Illustration of different GVNP shapes' ability to resist collapse. The narrow cylindrical GVNP (left) is able to resist pressure that flattens a spindle-shaped GVNP (right). Based on electron micrographs from [1].

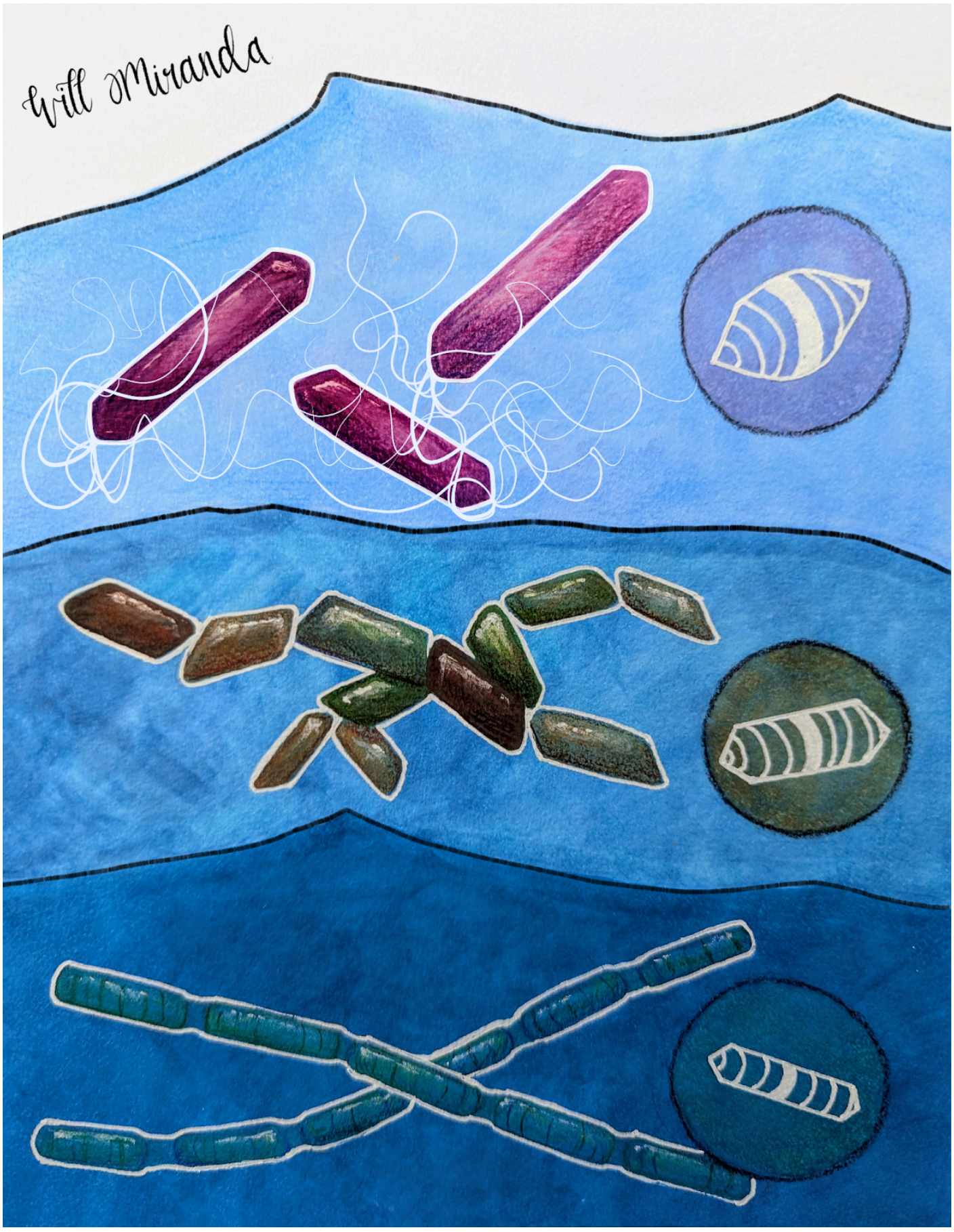


Fig. 5. Artist's rendition of series of different prokaryotes with GVNPs. From top to bottom, Haloarchaea, *Pelodictyon clathratiforme*, and *Planktothryx rubescens*. These organisms are not necessarily found in the same environment. Based on [2, 3, 4, 5, 6]. © 2024 Willow Miranda

Originally, scientists believed that GVNPs were made of only one protein: GvpA. While GvpA is the main structural protein of GVNPs, scientists would soon discover another protein called GvpC on its surface. Today we know that there are over a dozen proteins involved in GVNP synthesis [7].

Fig. 6. Artist's rendition of a gas vesicle nanoparticle. GvpA forms the main body of the GVNP, while GvpC attaches to the surface of the GVNP along GvpA's spiral ribbing, strengthening the gas vesicles. © 2024 Willow Miranda

It is believed that GVNPs begin forming at the ends and then add proteins to the center of the organelle, but this has not been confirmed [1].

There are 13 or 14 genes that control GVNP synthesis in haloarchaea. These genes are called *gvp*M, L, K, J, I, H, G, F, E, D, A, C, N, and possibly O. In NRC-1, the model organism used for haloarchaea, these 14 genes are located in clusters on plasmids, small extrachromosomal molecule containing DNA in microorganisms. Most of the studies have been done on a plasmid/mini-chromosome (a structure similar to a plasmid which contains essential genes) called pNRC100. They are organized into two operons, that is, sections of multiple genes that share a promoter and are transcribed together. These operons are transcribed divergently, meaning that they are transcribed in opposite directions from the same promoter region. The leftward operon contains the genes *gvp*D, E, F, G, H, I, J, K, L, and M. The rightward operon contains *gvp*A, C, and N. The leftward operon is believed to encode proteins for the early stages of GVNP synthesis, while the rightward operon encodes structural proteins [7]. This is why knocking out the *gvp*C or *gvp*N genes results in smaller gas vesicles and knocking out genes in the leftward operon interferes with the cell's ability to create vesicles. Although *gvp*O is located downstream of *gvp*N, research suggests that *gvp*N is the last gene on the rightward operon [8]. This supports the hypothesis that *gvp*O has no effect on gas vesicle synthesis.

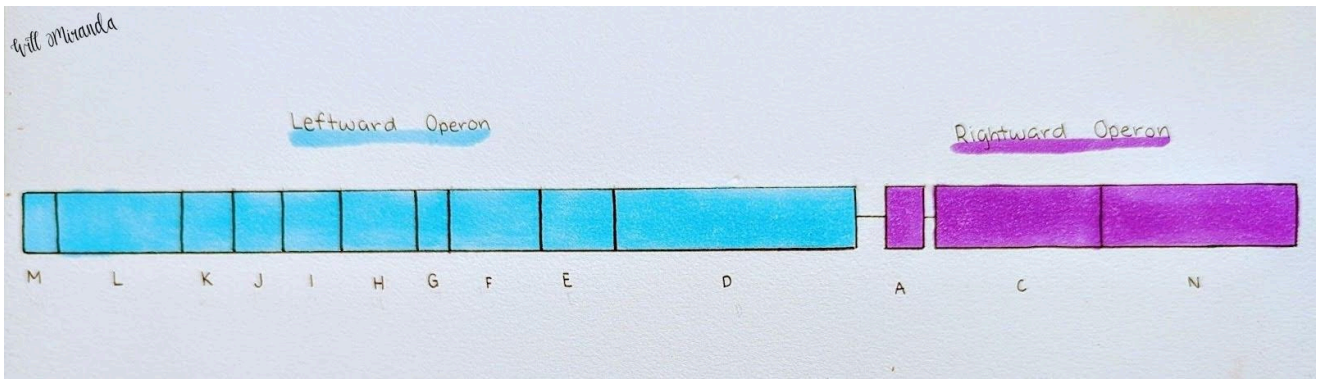


Fig. 7. To-scale representation of the two GVNP operons on pNRC100. Leftward operon is in blue, rightward operon is in purple. Based on [8]. © 2024 Willow Miranda

Of the 13 GVNP genes, at least 10 of them are necessary for GVNP synthesis. This table illustrates the effects of disabling every gene except *gvp*A via insertion mutations. Normal colonies containing gas vesicles appear a milky pink color, while colonies lacking GVNPs appear a translucent reddish orange [7].

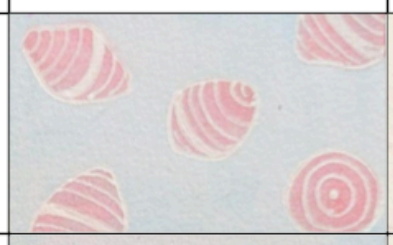
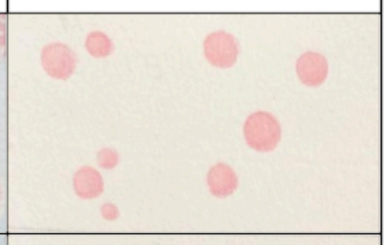
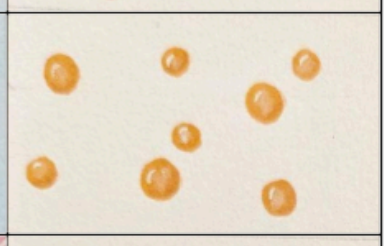
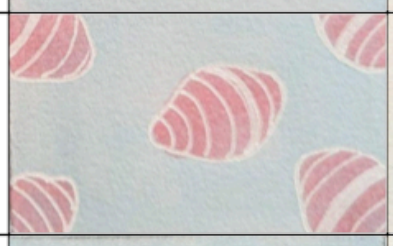
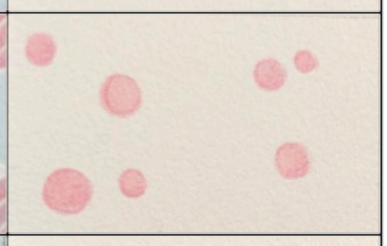
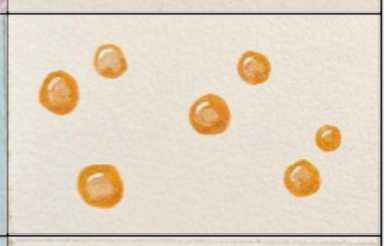
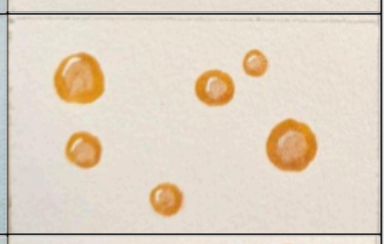
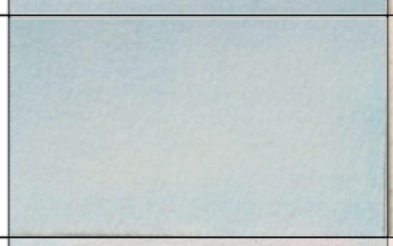
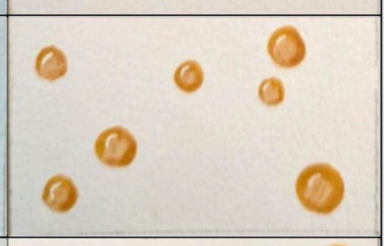
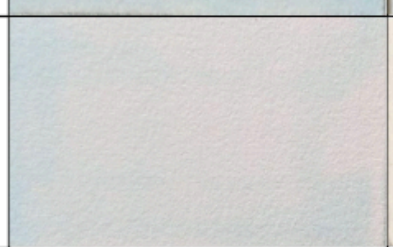
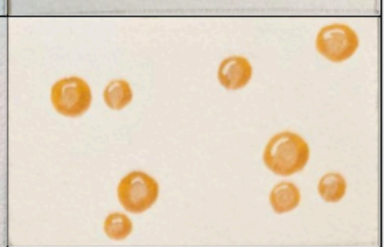
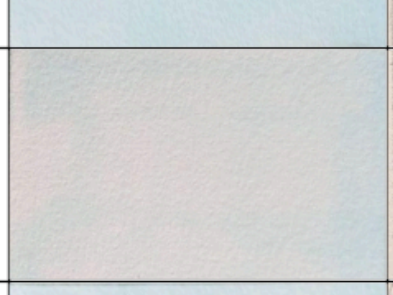
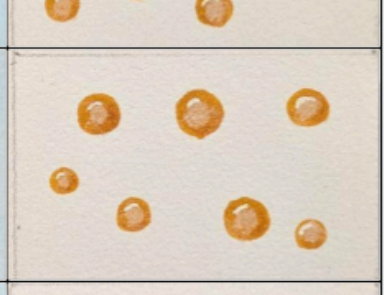
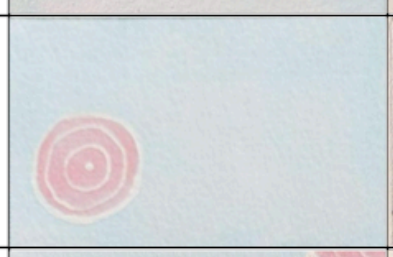
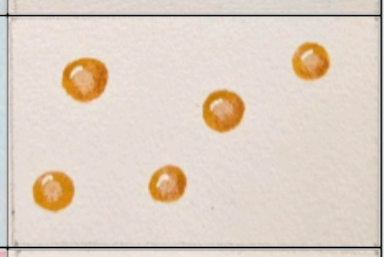
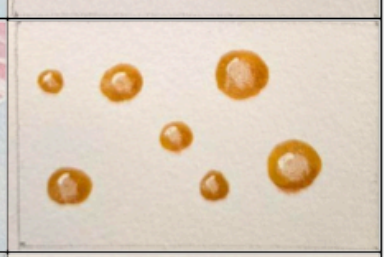
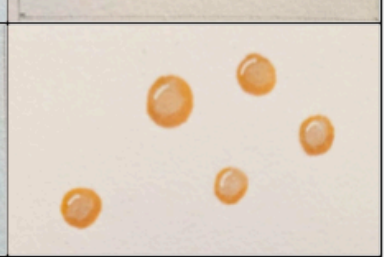
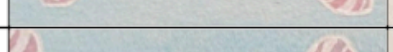
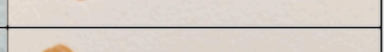
Gene Mutated	Phenotype	Effect on GVNP Production	GVNPs	Colonies
NRC-1 (wild type)	Vac^{+}	Normal GVNP synthesis		
SD109 (all genes removed)	Vac^{-}	No GVNP synthesis		
gvpM	Vac^{+}	Normal GVNP synthesis		
gvpL	Vac^{-}	No GVNP synthesis		
gvpK	Vac^{-}	No GVNP synthesis		
gvpJ	Vac^{-}	No GVNP synthesis		
gvpI	Vac^{-}	No GVNP synthesis		
gvpH	$Vac^{\Delta-}$	Greatly reduced GVNP synthesis		
gvpG	$Vac^{\Delta-}$	Greatly reduced GVNP synthesis		
gvpF	Vac^{-}	No GVNP synthesis		
gvpE	$Vac^{\Delta-}$	Greatly reduced GVNP synthesis		
gvpD	$Vac^{\Delta-}$	Greatly reduced GVNP synthesis		
gvpC	$Vac^{\Delta-}$	Contains small GVNPs		
gvpN	$Vac^{\Delta-}$	Contains small GVNPs		

Table 1. Different strains of NRC-1, their phenotypes, their effects on GVNP production, and illustrations of their GVNPs and colonies. Based on [7]. © 2024 Willow Miranda

In order to determine if the observed changes were the result of deactivating the mutated gene or the result of an insertion sequence interfering with the other genes downstream of it, the haloarchaea were once again mutated. Nearly the entire insertion sequence was deleted, leaving only a handful of inserted codons. For the most part the results were unchanged, with two notable exceptions: *gvp*D and H. In these two cases, normal GVNP synthesis was restored [7].

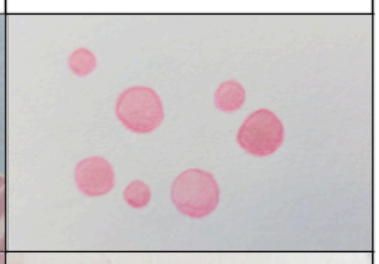
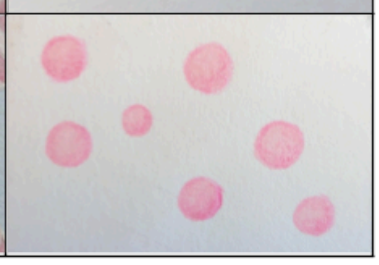
Gene Mutated	Phenotype	Effect on GVNP Production	GVNPs	Colonies
gvpD	Vac$^+$	Normal GVNP synthesis		
gvpH	Vac$^+$	Normal GVNP synthesis		

Table 2. Depiction of *gvpD* and *gvpH*'s return to wild type GVNPs after deletion of most of the cassette. [7]. © 2024 Willow Miranda

In addition to being completely unique in nature, gas vesicle nanoparticles have amazing medical potential. There are several properties that make GVNPs useful for biomedical applications. The first such property is that they are easy to purify. That is, they are simple to isolate from their cells (DasSarma & DasSarma, 2021).

When a cell lyses, or bursts, the GVNPs float to the top of the solution, where they can easily be collected while the rest of the cell sinks. Scientists can procure large quantities of purified GVNPs through hypotonic lysis and centrifugation. Halobacteria are salt-loving extremophiles, and they grow in briny water that can be nearly completely saturated with salt.

Water diffuses through a membrane from an area of lower solute concentration to one of higher concentration. Halobacteria's briny waters have an extremely high solute concentration, and their cells have adapted to match such conditions. In high salinity conditions, haloarchaea are isotonic to their surroundings, meaning that the solution inside their cells has the same solute concentration as outside their cells. When the cells are isotonic, water flows in and out of the cells at the same rate, and the organisms are healthy.

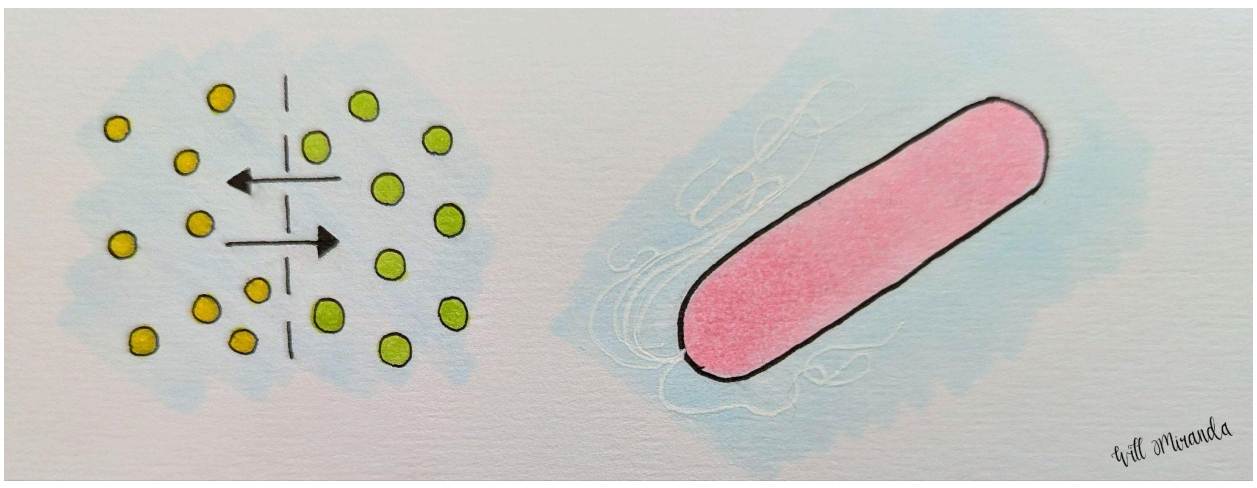


Fig. 8. Depiction of osmotic equilibrium and a *Halobacterium* sp. NRC-1 in an isotonic solution. © 2024 Willow Miranda

However, if the surrounding water's salt concentration drops, the cell is suddenly hypertonic to its surroundings, and water flows into the cell at a much faster rate than it exits. This causes the cell to swell and lyse. Most halophiles will lyse in solutions with a salinity of less than 1 to 1.5 molarity (M) [9].

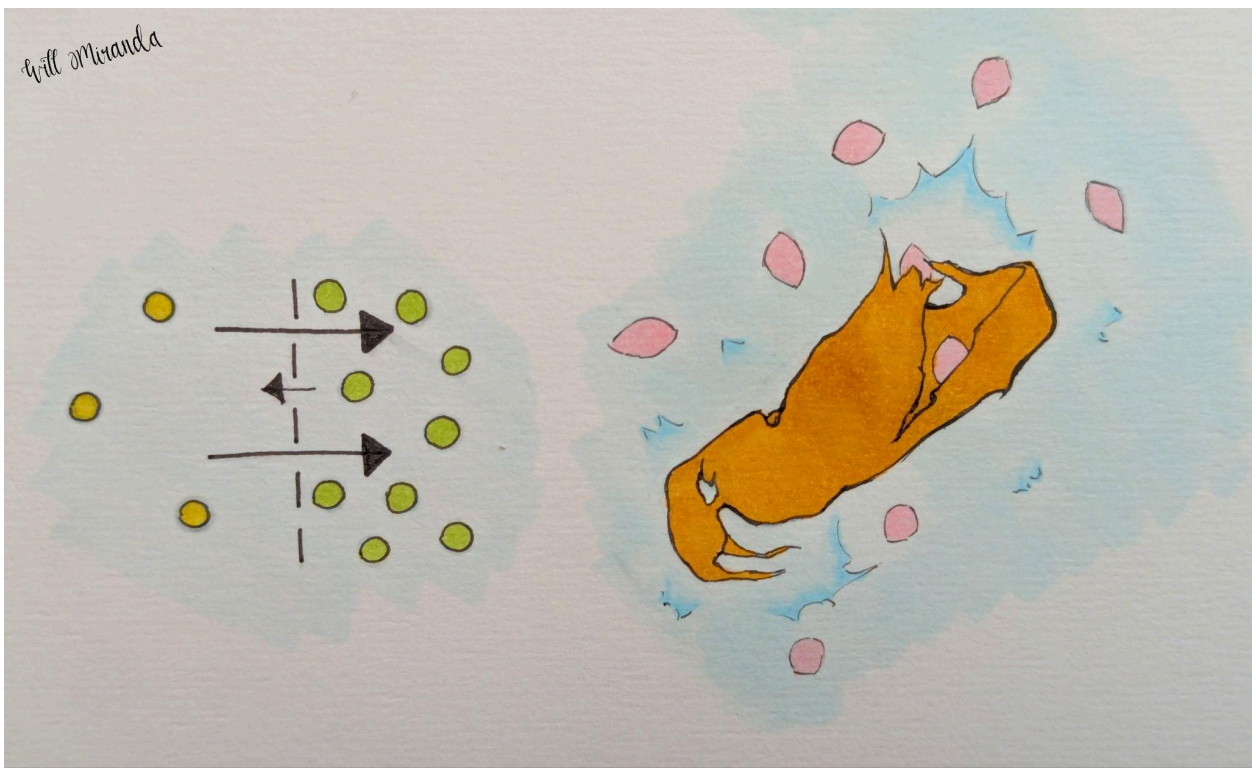


Fig. 9. Depiction of haloarchaeal cell in hypotonic solution and cell lysis, with purified GVNPs escaping. © 2024 Willow Miranda

After the cells lyse, scientists use centrifugation to speed up the flotation of GVNPs [10]. In centrifugation, solutions are placed in centrifuges and spun at high speeds to separate the different parts of the cell by density. This makes the GVNPs float to the top and the rest of the dead cells to form a pellet at the bottom of the centrifuge tube.

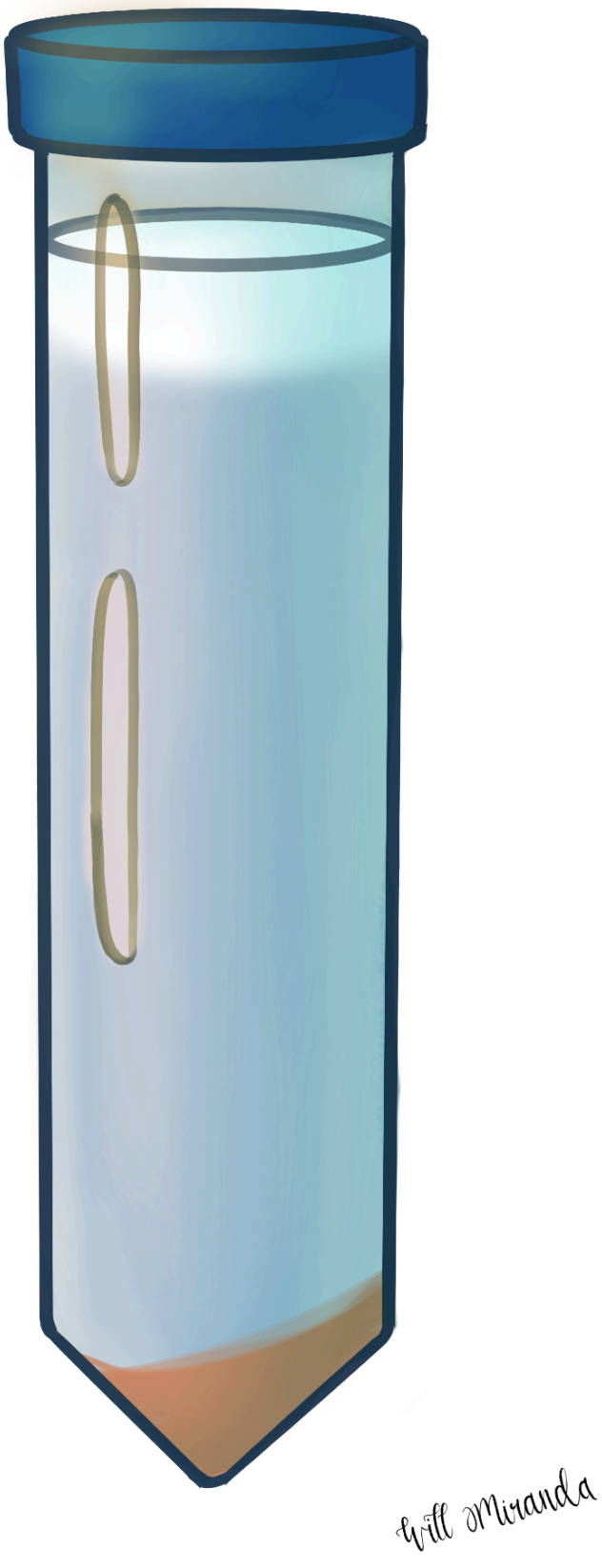


Fig. 9. Depiction of centrifuge tube with floating GVNPs and pellet from the rest of the cell. Based on [11]. © 2024 Willow Miranda

The second important property of GVNPs is that they are nontoxic to animals [12]. Their lack of toxicity means that they may be able to be injected into humans with no ill effects.

Thirdly, GVNPs are extremely stable. This stability means that they can be stored for a long time without expiring, and they do not need to be stored in the cold. Additionally, GVNPs are able to confer stability to compounds attached to them, which brings us to the fourth important property of GVNPs.

The fourth and most important property for GVNPs' medical applications is the fact that other proteins can be fused to GVPC and attached to the surface of GVNPs. This ability is central to GVNP's usage in medicine.

Proteins can be attached to GVNPs by modifying GvpC proteins. *gvp*C is able to accommodate modifications at the end of its coding sequence, resulting in functional GvpC proteins with foreign proteins displayed at their ends. These are called fusion proteins

as they are created by fusing parts of two separate genes. Furthermore, wild-type GVNPs are not fully saturated with GvpC, meaning that GvpC fusion proteins can be synthesized outside of the organism and will bind to the GvpA [13].

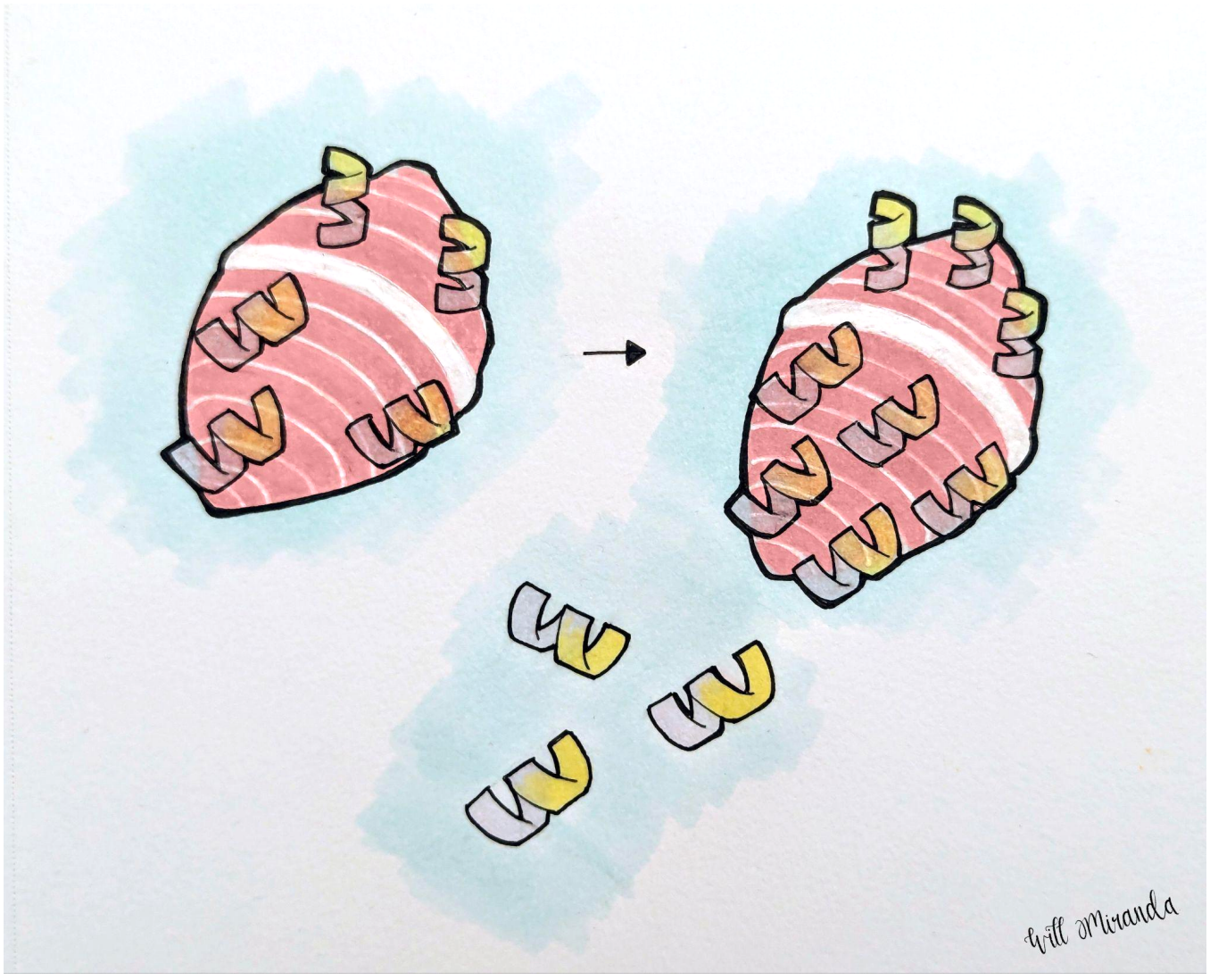


Fig. 10. Illustration of wild type GVNP (left) with added GvpC (center) fusing to it (right). © 2024 Willow Miranda

One method involves using *E. coli* for cloning and production of GvpC fusion DNA and proteins and attaching those fusion proteins to wild-type GVNPs [13]. Another involves deleting the *gvpC* gene from a strain of NRC-1 and creating a plasmid containing a *gvp*C fusion gene. The plasmid is then introduced into the NRC-1, and it begins creating GVNPs with the modified *gvp*C gene on its own [10].

GVNPs are exceptionally well-suited to vaccines. They are very small and nontoxic, making it safe to inject them. Additionally, creating GvpC fusion proteins lets them be modified to display antigens. Antigens are biomolecules that can bind to specific antibodies. These antibodies surround and bind to the cell displaying the antigens, creating a clump that stops infectious particles from interacting with other parts of the body and makes it easier for white blood cells to ‘eat’ them via phagocytosis. Antigens are usually made of proteins, peptides, sugars, or some combination thereof.

In most vaccines, the injection contains viral antigens. The body then reacts to them, creating long-lived antibodies for that disease and memory cells, which remember antigens so that the body can fight them off in the future. Attaching viral antigens to GVNPs allows them to function in this manner.

In addition to being bioengineerable for antigen display, GVNPs are self-adjuvanting. Adjuvants are components of vaccines that strengthen the immune response to antigens in vaccines. They are not typically necessary for vaccines that contain whole weakened or killed viruses since those usually have natural adjuvants. However, most modern vaccines contain only small portions of germs, such as their proteins. In these cases, adjuvants are used to strengthen and provoke an immune response specific to the virus type [14]. This is what allows such vaccines to confer immunity.

Despite the fact that GVNP vaccines also contain only the surface proteins from viruses, they do not need an external adjuvant. Instead, the GVNPs serve as both adjuvants and antigen carriers. Not only does this simplify the process of vaccine creation, but it also decreases the negative effects of vaccines while maintaining full effectiveness. Both weakened viruses and added adjuvants are responsible for increased reactogenicity, common and expected adverse reactions such as swelling or aching [14], and weakened viruses can sometimes be unsafe for immunocompromised people. On the other hand, vaccines containing dead

germs cause less reactogenicity but do not provoke a very strong immune response, and they are less effective overall [15]. However, GVNP vaccines are able to increase immunogenicity without affecting reactogenicity [12].

Furthermore, GVNPs' exceptional stability significantly extends the shelf life of any vaccines made with them, and they do not need to be refrigerated to stay functional [12].

Because of our inability to completely determine the composition of GVNPs, there are currently no GVNP vaccines approved for use on humans. However, they have tremendous potential to advance vaccinology.

In addition to creating GVNPs that display antigens, it is also possible to make GVNPs that display antibodies. One experiment used *E*. *coli* to replicate fusion proteins made of GvpC and immunoglobulin-binding streptococcal protein G, also known as SPG. SPG has multiple binding sites for immunoglobulins, another word for antibodies, as well as a site for albumin. This experiment attached these fusion proteins, named GvpCGBs, to the spiral ribbing of GVNPs. They then introduced monoclonal antibodies, proteins made in labs that act like normal antibodies, to check if the binding sites on the GvpCGBs were functional, proving that GVNPs can be modified to display antibodies [13]. In theory, this method could allow GVNPs to display many different antibodies, including potentially multiple antibodies on one GVNP.

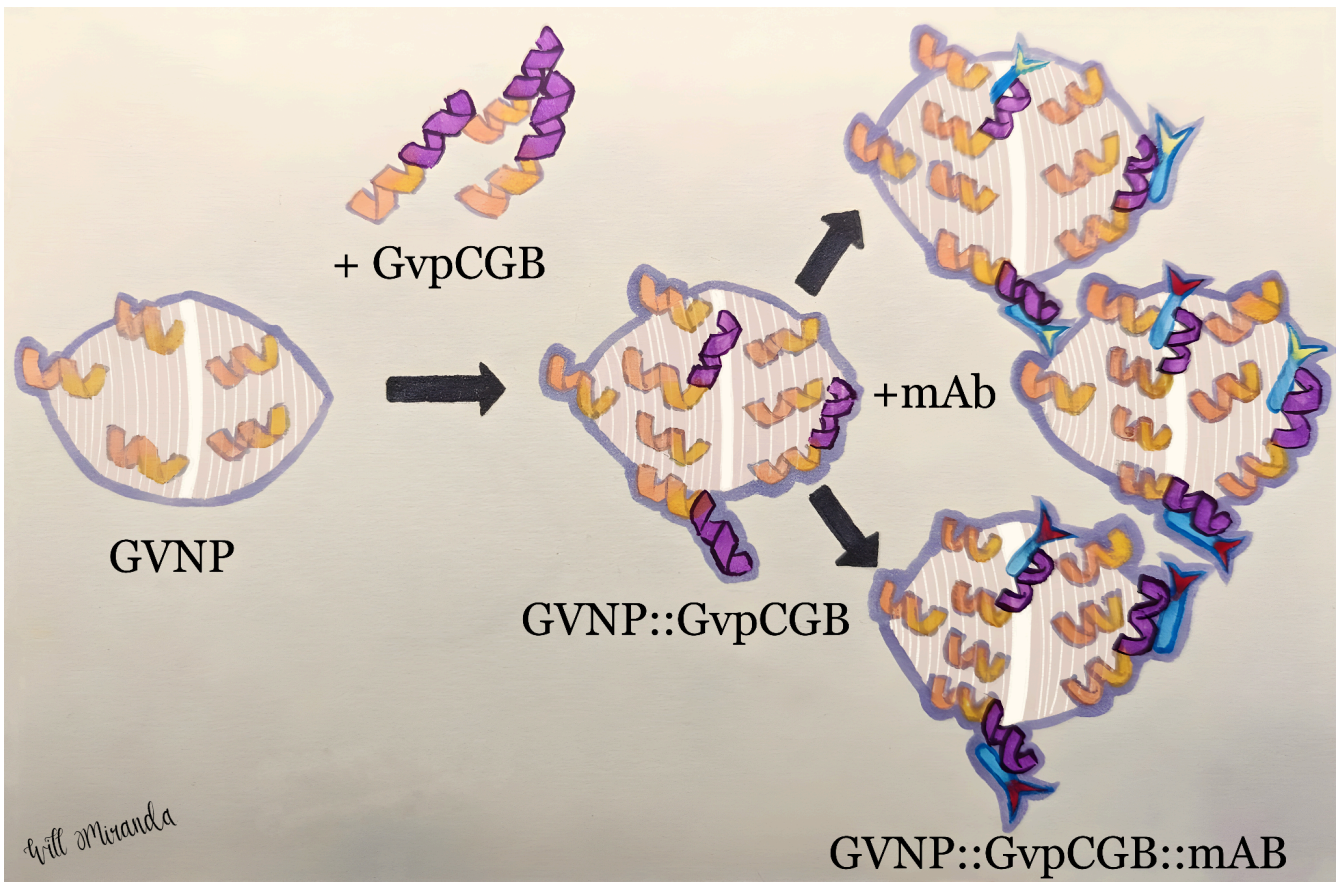


Fig. 11. Illustration of method to display antibodies on GVNPs. Pink and yellow spirals are wild-type antibodies, purple spiral is the SPG section of recombinant GvpCGB proteins, forked blue and yellow or red structures are different types of monoclonal antibodies. Recombinant GvpCGB fuses to the surface of the GVNP, forming GVNP::GvpCGB. Monoclonal antibodies (mAB) attach to the GvpCGB, forming GVNP::GvpCGB::mAB, which can be used for therapeutic treatments. Based on [13]. © 2024 Willow Miranda

Beyond that, it is possible to modify GVNPs with many other proteins and therapeutic agents. For instance, *gvp*C can be fused with a gene for bactericidal/permeability-increasing protein, BPI, which can negate the effects of Gram-negative bacteria through an interaction with the liposaccharides, LPS, that form a part of the bacteria's outer membrane [16]. Gram-negative bacteria can cause infections, and if they reach the bloodstream the LPS on their surface can trigger an immune response that causes an inflammatory response. This response can be exceedingly dangerous, often resulting in endotoxic shock and sepsis. Sepsis is the result of the body's immune response causes inflammation that damages vital organs. It can lead to organ failure, extreme tissue damage, and often death. It is highly lethal, with a mortality rate of somewhere between 28 and 50% [16]. One of the best ways to neutralize these endotoxins is through the use of BPI. Recombinant DNA containing sections of human BPI has been shown to be very effective in producing anti-inflammatory responses in humans, thereby counteracting the effects of endotoxic shock. However, this rBPI21 has a very short shelf life and is exceptionally expensive, making it unavailable for widespread use [16].

These issues could potentially be circumvented by fusing BPI and GvpC to create GVNPs displaying bactericidal/permeability-increasing proteins. GVNPs, unlike rBPI21, have a long shelf life, and they are easier to produce. Additionally, they have no inflammatory effect on animals, and they have been shown to stabilize foreign proteins, making them a prime candidate for administration of recombinant BPI.

While there have been no clinical trials with these recombinant GVNPs in humans, there have been experiments involving mice. In these trials, the recombinant mouse BPI and GcpC proteins, mBPIN-GVNP-C3 fusion proteins did not have any anti-inflammatory effects on their own. However, when attached to the surface of GVNPs, the stabilizing effects of GVNPs allowed the mBPIN-GVNPs to neutralize septic shock in mice, provided that it was administered before the LPS [16]. These results show promise for the possible therapeutic treatment of endotoxic shock in humans.

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