# Helix

## Milton Academy's Science Journal

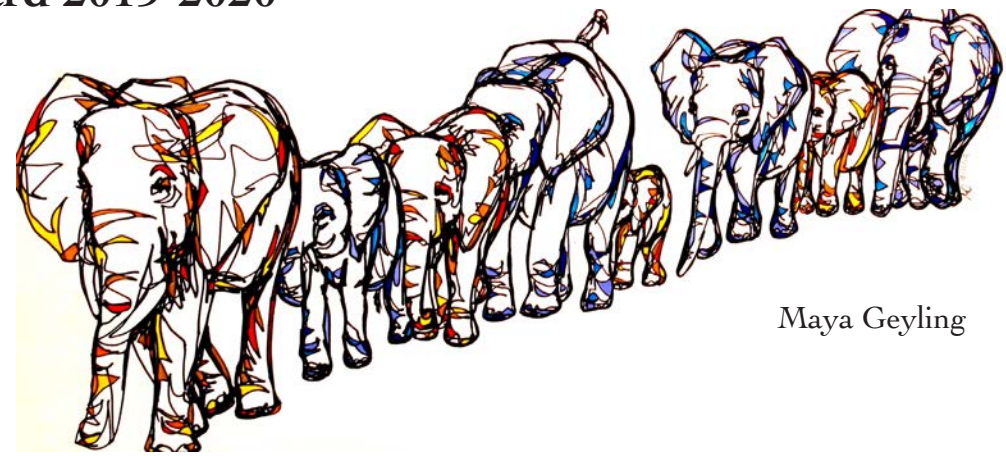
Winter 2020

# Contents



## Helix Editorial Board 2019-2020





Maya Geyling

**Special Thanks to. . .**
Mr. Edgar, Dr. Richards, Mr. Reilly, Ms. Pedersen, Mr. Kernohan, and Ms. Lockwood

# No More DSBs?

## How Base Editing Takes CRISPR to a New Level

by Anna Yang '23

Gene editing is a process of modifying DNA to correct or induce mutations. It has the potential of curing many genetic diseases such as Sickle-cell anemia, color blindness, Tay-Sachs disease, and some types of cancer. These diseases are caused by point mutations, which are mutations in the DNA sequence that involves a change in a single base pair. Consequently, point mutations may alter the amino acid sequence of the translated polypeptides and impact its functioning. Currently, the existing genomic editing technologies, such as the CRISPR-Cas9 system, edit the DNA base sequence through an engineered nuclease. CRISPR, for example, was originally a defense mechanism used by bacteria against foreign pathogens, but was engineered in recent years for targeted gene editing. The enzyme Cas-9 acts as a nuclease, and was associated with a guide RNA that locates the target sequence. Nucleases such as Cas-9 induce double-stranded breaks (DSB) in the sugar-phosphate backbone of DNA as the first step to correcting mutations. DSBs initiate one of two responses, non-homologous end joining (NHEJ), or homology directed repair (HDR). NHEJ is initiated when a homologous strand is absent. Random insertions and deletions (indels) were formed in the locus where the sugar-phosphate backbone was broken by the Cas-9 protein, disrupting or inactivating the gene. The cell uses the mechanism of HDR when a homologous strand is present, which uses an intact homologous strand as a template to complete the missing bases. Although CRISPR is the most widespread genome editing technique available, this method may lack in efficiency and specificity. When NHEJ and HDR compete for DSB repair, indel formation always occur to some extent (1). This leads to gene disruption or inactivation. To prevent the random insertion and deletion of nucleotides in response to a DSB, the target base must be directly converted without cleavage of the DNA strand. This goal was achieved by the recently developed technology of base editing.

A study by Komor et al. provides a gene editing technique called base editing that directly and irreversibly converts a base pair into another, increasing the accuracy and efficiency of HDR while suppressing indel formation from NHEJ. They synthesized an enzyme capable of in situ converting a C into a U, which has the same base pairing property of a T. By inactivating the protein Cas-9 (dCas-9) through Asp10Ala and His840Ala mutations (2), the enzyme loses the ability to induce DSBs, but retains its DNA binding ability in conjunction with a guide RNA that locates the target gene. Then, dCas-9 is linked to a cytidine deaminase, which directly converts the C into a U by atomic rearrangement of the nucleotide. Under the tested conditions, the enzyme rat APOBEC1 shows the most promising deamination activity compared to other cytidine deaminase enzymes (3).

The G:U discrepancy in the target locus can be permanently converted to the A:T pairing through the innate cellular function of DNA repair and replication. DNA repair is a process where a protein complex recognizes a mismatch in base pairing, which is then corrected by a poly-

merase enzyme and sealed by DNA ligase. The maximum efficiency of this method is 50% yield of conversions (3), and this suboptimal efficiency was a result of the cellular response of eukaryotic mismatch repair (MMR), which tends to repair the newly synthesized strand by recognizing its nicks before being sealed by DNA ligase (4). To prevent the MMR process from converting the edited U back to a C, the enzyme BE3 (5) was introduced to nick the unedited strand to simulate the newly synthesized strand for a higher probability of MMR repair (see Fig.1a). By inducing nicks, BE3 is used to manipulate MMR to target the original strand, thereby completing the substitution. With MMR favoring the unedited G, the C:G point mutation could be corrected to A:U. The A:U base pair is then permanently converted to A:T, the desired outcome, through DNA repair and replication. This method of nicking the target strand produces the base editor protein complex (BE3, APOBEC–XTEN–dCas9(A840H)–UGI), augmenting the efficiency sixfold, while resulting in a mere 1.1% average indel formation rate (6). Results confirm that this base editing technology has a significantly higher efficiency than Cas-9 mediated HDR (see Fig.1b), with substantially fewer indels. Fig.1: a) BE3 redirects the prefered repair mechanism of cellular base-excision repair by nicking the target strand, resulting in the desired outcome of base editing. b) Percentages of successful C:G→A:T repair, showing the highest efficiency with the BE3 base editor (3). By avoiding double-stranded breakages, base editing improved in efficiency and reduced indel formation rate in the repair of point mutations. It has the potential to be utilized in a clinical setting to

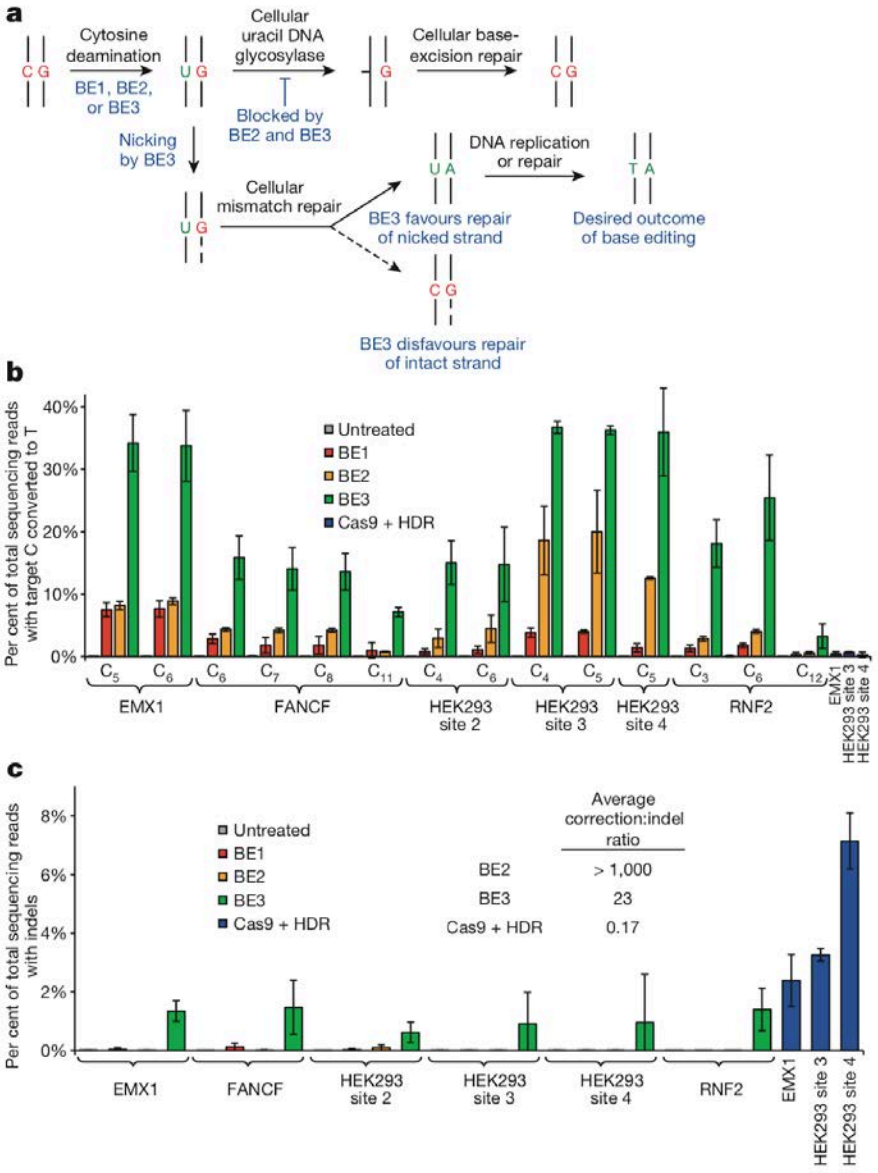



Fig.1: a) BE3 redirects the prefered repair mechanism of cellular base-excision repair by nicking the target strand, resulting in the desired outcome of base editing. b) Percentages of successful C:G→A:T repair, showing the highest efficiency with the BE3 base editor (3).

cure genetic diseases caused by a single point mutation of A:T→C:G. Many inherited diseases resulting from a single T→C point mutation experienced advancement due to base editing. For example, sickle cell anemia was caused by a point mutation in the hemoglobin gene substituting a glutamic acid with valine in the beta-globin polypeptide. Effects on the protein level are hemoglobin molecules that form clumps; this leads to deformed red blood cells with a sickle shape, and a decreased affinity for oxygen. If an individual contains both copies of the mutated gene, sickle cell disease occurs, with many medical implications on the organismal level. Base editing already shows a significant advancement in the treatment of sickle cell anemia and many similar diseases, by correcting the underlying point mutation. This technology of base editing was one of the many newly developed methods of direct base editing that took the CRISPR technology to a new level. Many more discoveries are in progress in the field of genetic engineering. One recent breakthrough is prime editing (7), which takes the concept of base editing (the direct conversion of nucleotides without double-stranded cleavage) and develops a safer and more efficient alternative. The potential of gene editing techniques such as base editing are endless in the scientific and medical fields. What do you think the next step would be?

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# The Stress Mechanism

by Miriam Zuo '20

It often feels like there's an endless stream of stressors: maybe it's time to take a step back.

By this point in the school year, most students have experienced stress, whether in the form of scrambling after deadlines, stressing about grades, or running out of time on a test. Perhaps unsurprisingly, this feeling is common: 79% of Americans report feeling stressed sometimes or frequently throughout the day (1). Yet, though the outward sensation of stress may be all too familiar, we are often less clear on its underlying causes, as well as its detrimental effects and how it may be mitigated.

Stress is the body's "fight-or-flight mechanism," a process that was evolutionarily useful in surviving dangerous and challenging situations (2). Though we no longer regularly encounter predators that threaten our survival, the modern world brings a host of stressors that continue to activate this mechanism. When a person faces a stressful situation, the "fight-or-flight mechanism" is activated by chemicals called hormones; in particular, it's affected by cortisol, adrenaline, and noradrenaline (2). Cortisol is the hormone most closely associated with stress, although it is also involved in other processes like regulating blood pressure and the sleep cycle (3). It is released by the adrenal glands as a result of chemical interactions along the hypothalamic-pituitary-adrenal (HPA) axis; this axis, as suggested by its name, involves the hypothalamus and pituitary gland in the brain, as well as the adrenal glands on top of the kidneys (4). The adrenal gland also produces adrenaline, otherwise known as epinephrine, which "makes the heart beat faster, increases blood flow to the brain and muscles, and stimulates the body to make sugar to use for fuel"; these effects are perceived as an adrenaline rush (5). The least well known of the stress hormones, noradrenaline (or norepinephrine) has similar effects as epinephrine but also has the additional ability to elevate blood pressure (6). Combined, these three hormones produce the symptoms of stress that many people commonly experience: faster breathing, increased alertness, anger, anxiety, and difficulty concentrating (2).

While the specific effects of stress vary between individuals, the overall impact of stress is negative, particularly when compounded

long-term. The American Psychological Association's first category of stress is acute stress, which is short-term stress; it induces a weaker immune system, difficulty sleeping, sweating, and fainting (2). The second form of stress is episodic acute stress, and it is associated with frequent stress triggers, such as having too many commitments; its effects are more severe, significantly contributing to elevated blood pressure and heart disease (2). A growing problem in our work-driven society, chronic stress occurs when someone lives in perpetual stress (2). This third type of stress is particularly harmful, and it results in a higher likelihood of suicide, heart attacks, and strokes (2). Furthermore, the constantly elevated levels of cortisol attributed to chronic stress are a potent factor in anxiety, depression, weight gain, and memory and concentration problems (3).

Given the severely detrimental impact of stress on our physical and mental well-being, we should be conscious of actively managing our stress levels. Several strategies may be helpful: exercise, reduced caffeine intake, a healthy diet, yoga, meditation, and breathing techniques (2). Searching for de-stressors – activities and actions that lower stress levels – and building support networks are also effective for many people (2). As a whole, though it's often challenging to find extra time, putting in effort to reduce your stress levels yields worthwhile health benefits in the short-term, but its primary value results from its long-term benefits. Therefore, practicing meditation or going on a long walk lead to benefits that outweigh the amount of time invested.

Potential stressors abound in everyday life, especially in rigorous, competitive environments like Milton Academy. Yet, stress is not inevitable: our habits and our stress-coping mechanisms largely determine our body's response to the challenges of our surroundings. Instead of passively accepting stress, we should strive to take control over it to live healthier lives.

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# Bayes' Theorem

by Nikhil Pande '21

Since many elements of life are left solely to chance, probability is an important element of predicting the future. But probabilities are always affected by the given conditions of the situation. Bayes' Theorem, discovered by Thomas Bayes and formalized by Pierre Laplace, serves as one of probability's fundamental theorems (1). The premise of this theorem involves incorporating initial conditions to predict a future outcome (2). Bayes' theorem reads:

$$P(A \mid B) = \frac{P(B \mid A)P(A)}{P(B)}$$

Source: https://wikimedia.org/api/rest_v1/media/math/render/svg/87c061fe1c7430a5201eef3fa50f9d00eac78810

The capital letters A and B represent specific events. This notation of P(A | B) means the probability that event A happens given that event B has already happened.

The formula relies on the presence of other factors to determine the probability of an outcome. This method of probability is called Bayesian probability, more generally meaning the probability using previous outcomes or conditions for the purpose of prediction (3). The method of Bayesian probability contrasts the method of frequentist probability, in which the probability of a certain event is derived from its mere frequency of occurrence. Frequentist probability has a simple probability formula of (number of outcomes)(number of opportunities) (4). In this way, frequentism assumes the randomness of all other factors, making it insufficient as a predictor for more complex phenomena.

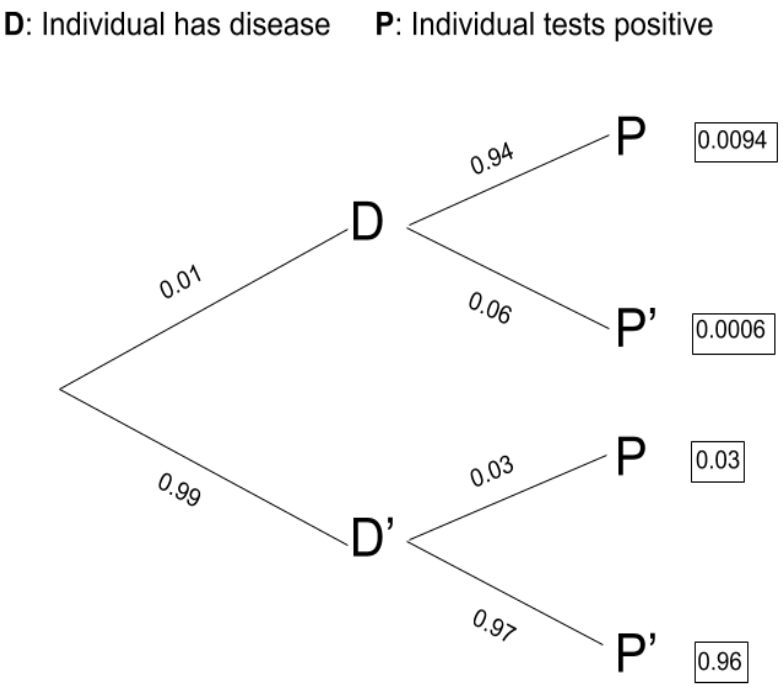
We can examine Bayes' theorem through a specific example. An individual goes into the hospital and undergoes testing to see if he has a certain disease. The probability that any individual in a total population has the disease is 1%. Additionally, the probability that the device used to test for the disease delivers a false positive (a positive reading for the disease when the patient does not have it) is 3%, and the probability of a false negative (a negative reading for the disease when the patient does have it) is 6%. If this specific individual ends up testing positive for the disease, what is the probability that he actually has it?

The following probability tree incorporates the information of the problem to output the probabilities of testing positive and negative for an individual that either has the disease or doesn't.

To find the probability of D given P, or P(D | P), we can simply divide the P(D and P) by the total probability of testing positive, which ultimately computes to about 24% with a calculation of 0.0094/0.0394. Of all patients tested positive, only 24% do indeed have the disease. This value may seem extraordinarily low, but is quite plausible when considering the miniscule rate of this disease in society.

For a test as important as a medical screening, the mere 24% chance of having the disease can look like a failure or incompetency of the testing device. However, for one thing, the chance of a positive test is extremely rare. If we look at the probability tree, the P(P)=0.0394, meaning that this scenario doesn't occur often. Furthermore, the uncertainty of only a 24% chance exists only for the probability of having the disease after testing positive, or P(D | P). For the probability of not having the disease when

testing negative, or P(D' | P'), the Bayes' theorem-derived value in fact delivers strong confidence in the test's result. From the tree, we can divide P(D' | P') by P(P' | D') + P(P' | D). The calculation of 0.9994/(0.96+0.04) comes out to a probability of 0.9994, a value that can be interpreted as a 99.94% chance that the patient doesn't have the disease after testing negative. The P(D' | P') value is equal to the numerator of the Bayes' theorem, which is P(D'| P')*P(D'). The denominator is equal to the ultimate probability of a negative test, P(P'). Thus, when we used Bayes' theorem for the accuracy of a positive test, it provided us with an uncertain 24% chance of having the disease, but when using it for negative tests, it gave us a very strong assurance of accuracy, a 99.94% change of not having the disease. Because of Bayes' theorem, professionals in all fields are able to incorporate given conditions into probability calculations, which can help bring more certainty into diagnoses and other decisions. This theorem can be used not only in regards to false positives, but also with the stock market, sports predictions, architecture, scientific predictions, and more. And through its versatility, it enhances the way we evaluate chance in this unpredictable world.

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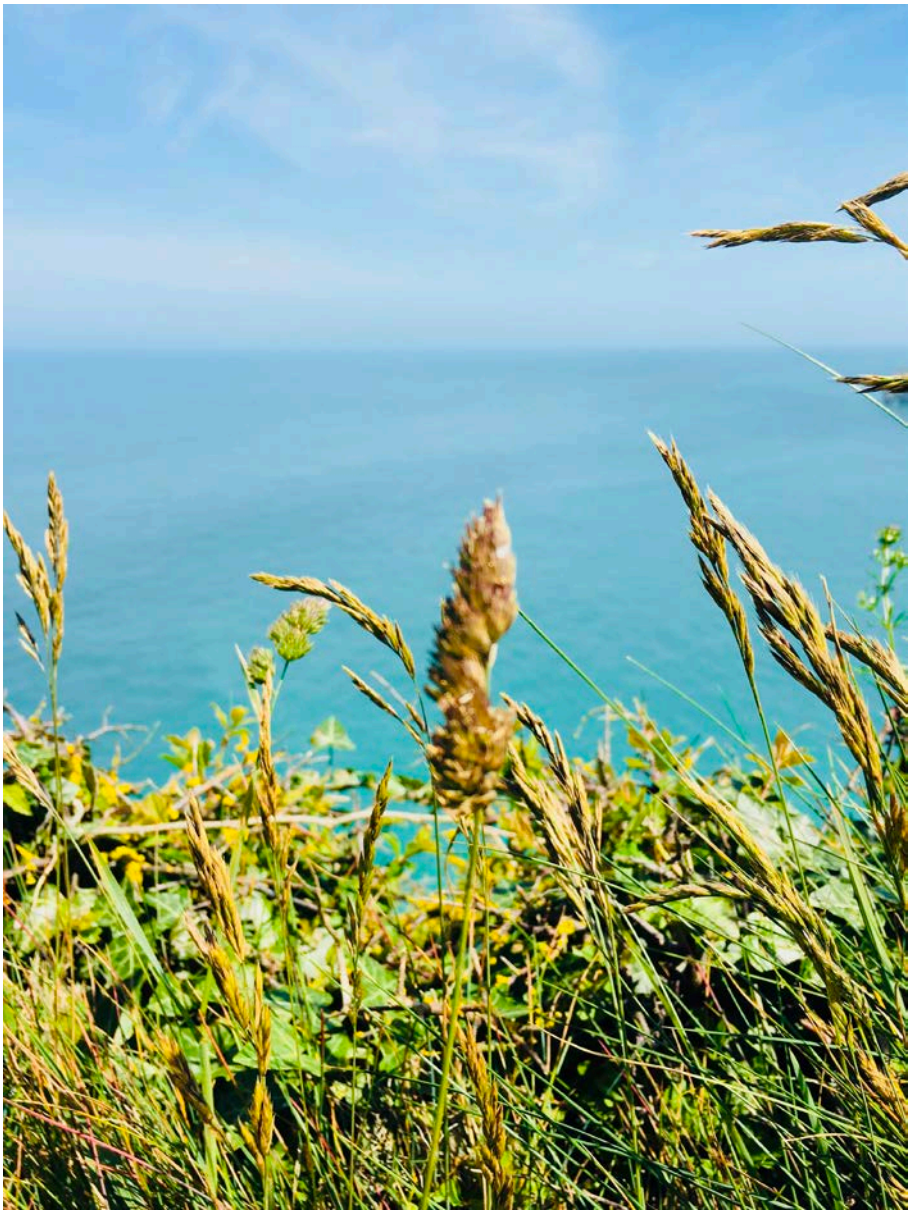
# Photography

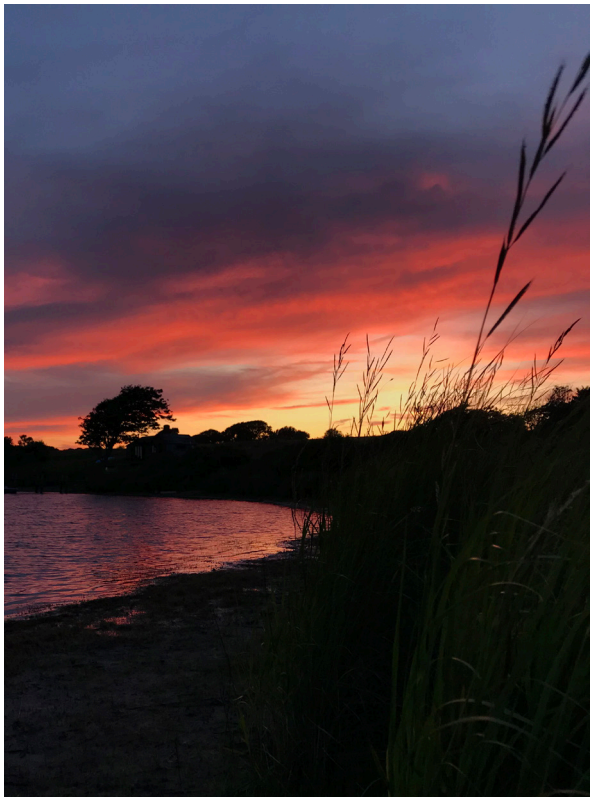
**Mya Lampley '22**

**Maya Geyling '20**



**Michaela Ocko ‘23**

**Kyra Geyling ‘23**



**Mya Lampley**

# Quantum Dots: Tiny Crystals Brightening Today's Technologies

by Wilder Crosier '21

Figure 1: Quantum dots exhibit fluorescence. From one vial to the next (left to right) the QDs increase in size by 10 nanometers. Smallest QDs emit violet light, largest QDs dots emit red light. Source: Wikimedia Commons https://commons.wikimedia.org/wiki/File:Quantum_Dots_with_emission_maxima_in_a_10-nm_step_are_being_produced_at_PlasmaChem_in_a_kg_scale.jpg

Some of today's largest innovations–more efficient solar panels, improved cancer imaging, and ever sharper TV displays–are coming from a very tiny system: the quantum dot. But what are quantum dots? And how are they such an effective and versatile technology? Quantum dots (QDs) are extremely tiny, semiconducting crystals, called nanocrystals. And when I say tiny, I mean really tiny–QDs range from 2-10 nanometers in diameter (about 10-50 atoms thick!) (1). The minute size and semiconductive properties of QDs give the crystals special optical properties that have made them such a distinctive technology.

The QDs' most employed property in modern technology is fluorescence: a property of some materials where they absorb light of one wavelength and reemit it as light of a different wavelength (2). When ultraviolet light shines on a quantum dot, an atom in the crystal will absorb a photon of this ultraviolet light. The energy from the photon causes an electron in the valence band (the atom's outermost energy level in its ground state) to jump into an excited state in the conduction band (a higher energy level) (3). The resulting energy difference (the jump) between the valence band and the conduction band is called the band gap (3). Electrons stay in the excited state for a very short period of time (between $10^{-9}$ to $10^{-8}$ seconds), and during this time they lose some of their energy. This energy loss is known as a Stokes shift (4). Now, the excited electron relaxes back down to the ground state, releasing a photon of visible light (lower in energy than the incoming UV due to the Stokes shift) with a characteristic wavelength (color) defined by the bandgap (2,3) (see figure 2). What's so special about QDs is that the physical size of the crystal determines the size of this electron bandgap, and therefore the color (or wavelength) of light emitted (5). Larger dots have smaller band gaps, so they emit longer wavelengths (lower energy light) towards the red end of the spectrum, while smaller dots emit shorter wavelengths towards the blue end of the spectrum (6). When QDs are synthesized, engineers can control the temperature and duration of a chemical reaction to make QDs of desired sizes, and therefore desired emission colors, without changing the material from which the dots are made (see figure 1) (10).

What makes these nanocrystals so eminent are their applications in modern technology which extend across the consumer, environmental, and medical

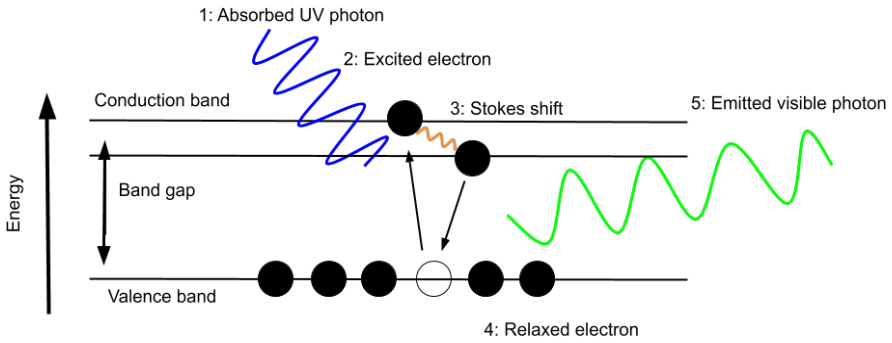



Figure 2: Schematic of fluorescence mechanism. An incoming UV photon excites an electron from the valence band, across the semiconductor's band gap, to the conduction band. The excited electron loses some energy by remaining in its excited state for a short period of time in what is called a Stokes shift. The now lower energy excited electron falls back to its ground state releasing a photon of visible light.

fields. Technology companies like Samsung and LG have created TV displays that use QDs to give their screens richer colors. In a traditional TV's LCD display, a blue LED shines through yellow phosphor to create white light, which can then be adjusted to any color by the red, green, and blue filters in each pixel (8). With a quantum dot display (QLED), instead of using phosphor to make white light, the blue LED shines through QDs with red and green sized band gaps. Because each color in a quantum dot LCD display is a pure red, green, or blue, the color coming through the filters is more pure (8).

Quantum dots also have the ability to greatly increase the efficiency of today's solar panels. Led Calderone writes for AltEnergyMag that quantum dot solar cells could be "at least three times more efficient than existing manufactured solar cells" (7). The sun emits light over the entire electromagnetic spectrum, but traditional solar cells struggle to capture light from the far infrared. Because engineers can produce QDs at desired sizes, each with a specific band gap, they can capture the infrared light easily (7).

Quantum dots also hold a lot of promise for medical imaging and drug delivery. Current research on cancer imaging involves binding quantum dots to antibodies and injecting them into the body. The antibodies will move about the body and then bind to cancer cells. Because the quantum dots fluoresce, doctors could use UV light to find and image cancer cells at early stages (9). The color tunability and high brightness of QDs makes them especially effective for cancer imaging over other fluorescing molecules. Researchers hope to eventually attach drug molecules to the antibodies and quantum dots for targeted drug delivery directly to the cancerous cells. The light coming from the QDs would allow scientists to monitor the delivery of the drug to the cancer (9). However, researchers are concerned with the toxicity of quantum dots; quantum dots today are composed of compounds with heavy metals, most commonly made of cadmium selenide or indium phosphide (10), and research so far has not fully clarified how QDs composed of these metals affect the body (11).

Quantum dots, with their fine-tunable band gaps for light emission, have found applications in technology across many fields. Now, when you come across a QLED TV, you can not only enjoy the pure colors but also understand the science behind these fascinating, tiny crystals.

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# A Ninth Planet in our Solar System!? Or Are Some Physicists Jumping Too Fast To Conclusions

by Luca Mostofi '21

Ever since Pluto's heartbreaking demotion to dwarf-planet, there have been eight planets in our solar system. However, recent discoveries in the outer reaches of the solar system (tens of billions of kilometers beyond Neptune) indicate that there perhaps could be an undiscovered ninth planet that orbits very distantly from our sun (1).

Supporters of this ninth planet theory cite several, otherwise inexplicable anomalies in our system that could be reasonably chalked up to the presence of this planet. Firstly, the unique elliptical orbits of certain objects in the Kuiper Belt (a ring of icy objects that encompasses the area beyond both Pluto and Neptune) could point towards this planet's existence (2). This potential revelation would account for the orbits of those Kuiper objects' all pointing in a generally similar direction (Figure 1) (1). A Cal Tech professor, Konstantin Batygin, also mentions that this theoretical planet would be roughly five times the Earth's mass (4). No other known planet in our solar system checks in at that mass; however, five earth mass is the single most common size of any planet that we have discovered in the universe (4). Thus, the ninth planet would account for our system's lack of a planet of that size by providing one. Perhaps the most damning piece of evidence in support of planet nine is the idea of a perpendicular orbit. In the Kuiper belt, there are a few objects that orbit perpendicular to the orbit of the planets in our solar system, and scientists say that the existence of planet nine could explain these abnormal orbit

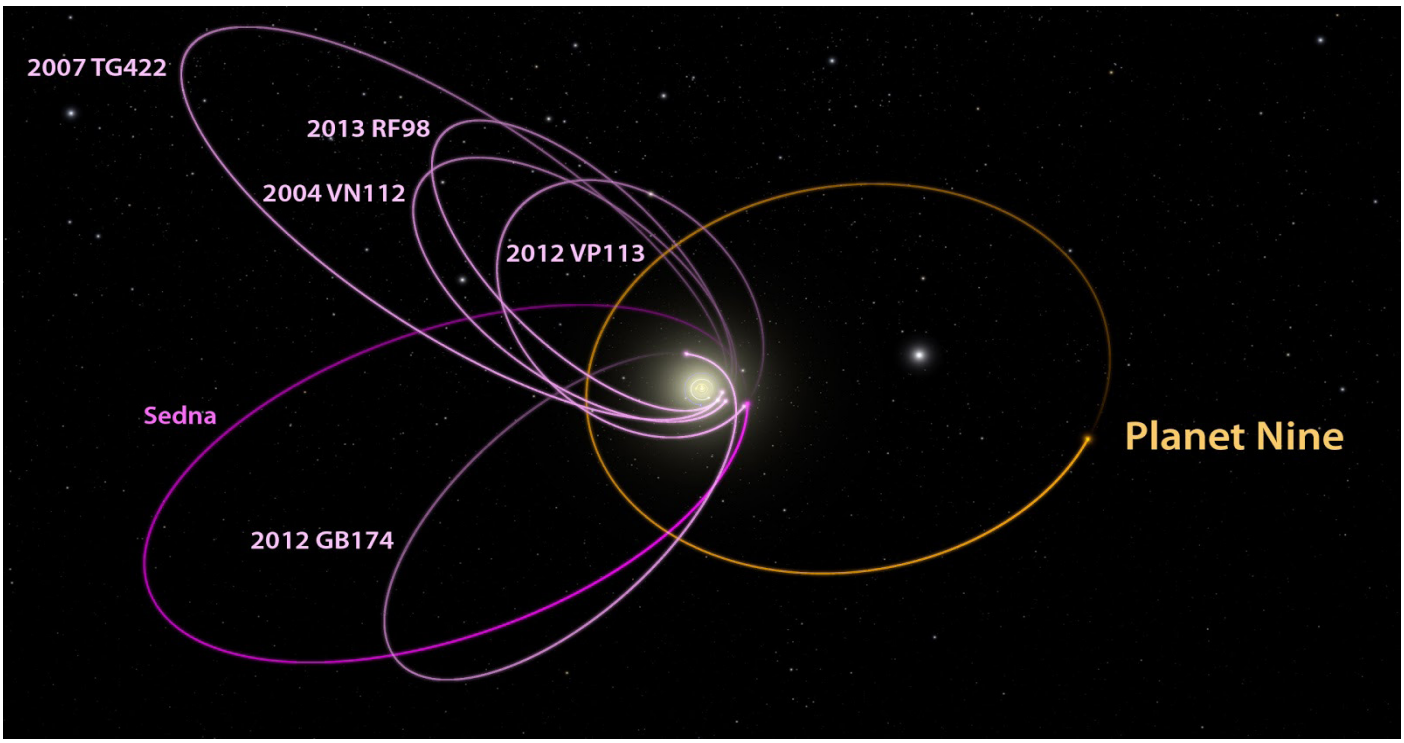


Figure 1

patterns (3). An object as substantial as planet nine would possess the considerable gravitational effects to account for the unconventional orbits of those Kuiper belt asteroids (3).

Detractors of the hypothesis ask a single, critical question: why hasn't anyone found it yet? The distant location of this planet - so far from the sun that it would take an estimated 10,000 years to complete a single orbital period - means that even the most powerful telescopes have a hard time picking up on it considering the lack of sunlight in those reaches of the solar system (3). Batygin notes that since 2017, the natural conditions (for example a calm atmosphere and no moon) that could theoretically make this planet visible (if you knew exactly where to look) have aligned on only two days. With the distance and conditions proving to be such a hinderance, Batygin believes finding it could take up to another decade (3).

A more far-fetched yet equally intriguing theory is that there is no ninth planet at all, but rather a primordial black hole in our solar system (5). A primordial black hole is a black hole that formed immediately after the big bang (6). Should one of these ancient black holes be hiding somewhere in our solar system, its mere presence would impact the solar system in the same ways as a ninth planet (7). However, finding it using standard planet-discovering procedures would likely be impossible because of its remarkably miniature size: a five earth mass black hole would fit snugly in the palm of one's hand (7).

While the theories of a truly ancient, baseball sized black hole or of an undiscovered, relatively nearby ninth planet would undoubtedly sell papers, they both seem implausible to me. Both hypotheses are burdened by the fact that neither have any concrete, visual evidence to cement their existence (such as a sighting). Regardless, whether there is a primordial black hole, a ninth planet, or nothing novel at all, our infinite universe continues to yield fascinating theories and discoveries.

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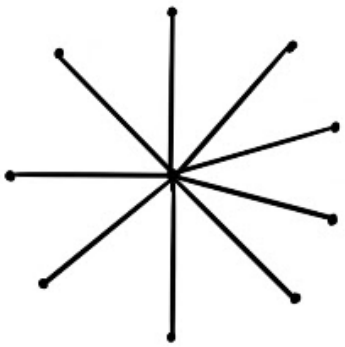
# Solving the Good Will Hunting Problem

by Madeleine Cesaretti '21

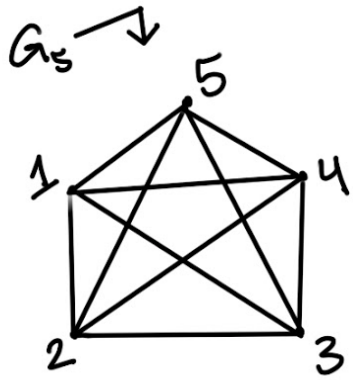
At the start of the Academy Award winning movie Good Will Hunting (Miramax Films, 1997), a professor at MIT presents his class with a problem. The professor writes the problem on the board and leaves it as a challenge for anyone to try and solve. Later that night, Will Hunting, a genius janitor at MIT, solves the problem and leaves his solution on the board. The next day, when none of the professor's students are able to claim credit for the solving of the problem, the professor writes another problem on the board -- this problem the professor claims took him and his colleagues two years to prove. Again, Will solves the problem but is caught in the act of doing so by the professor, and thus the events of the rest of the movie unfold. What is this "impossible" problem though? How can we go about solving it? The problem posed is as follows:

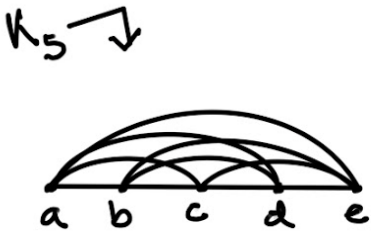
## Draw all homeomorphically irreducible trees of size n = 10.

Before we get to solving this question, we need to define a few of the words in there. First off, the trees being described in this problem are not trees in the most traditional sense of the word, but rather they are connected graphs with no cycles -- meaning that they consist of connected vertices and edges but one can't start and end at the same point without going over the same edge twice. "N labeled vertices" refers to the number of vertices in the tree and thus "size n = 10" tells us that these trees will be made of 10 vertices and from that we know there will be 9 edges as that is the highest number of edges that can exist off 10 vertices and still be connected. below is an example of one graph with n = 10. "Homeomorphic" means that within the comparison of multiple graphs there does exist a one-to-one correspondance between the vertices in one graph to that in another -- meaning that the vertices of one graph are adjacent to vertices of another graph. On the next page are two examples of graphs that are homeomorphic to each other.



n = 10



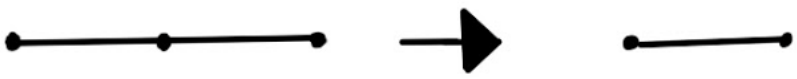

As you can see, vertices in $G_5$ and $K_5$ correspond to each other in the following way and are thus homeomorphic to each other:

| $G_5$ | $K_5$ |
|---|---|
| 1 | a |
| 2 | b |
| 3 | c |
| 4 | d |
| 5 | e |

corresponding vertices in graphs $G_5$ and $K_5$

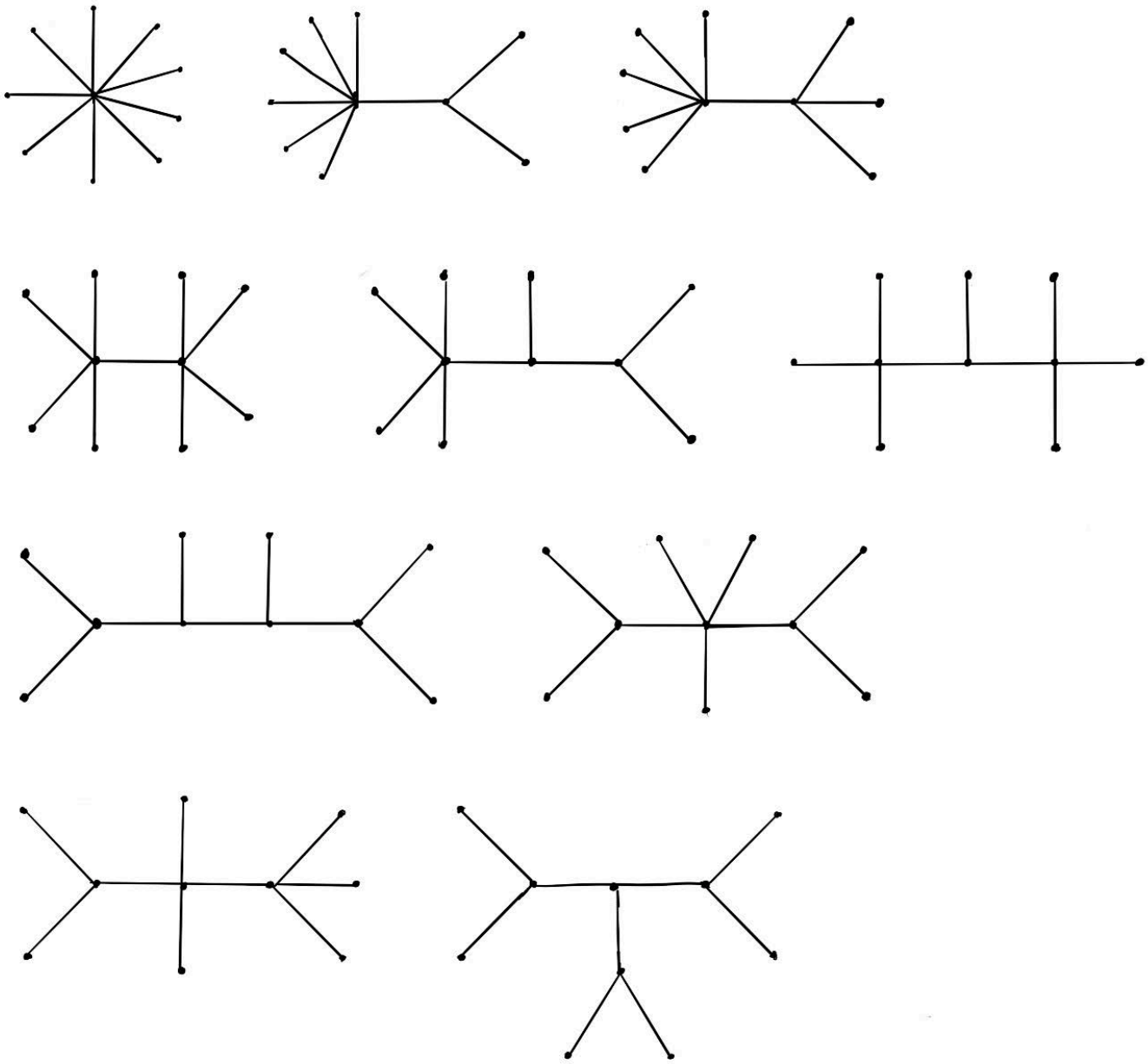
"Irreducible" indicates that in a graph there exists no vertices of degree 2; the degree of a vertice refers to the number of edges connected to a specific vertice. Right is an example of a graph that has a vertex of degree 2 that is then shortened to be irreducible.

I first encountered this problem watching Good Will Hunting with my friends, but I didn't pursue it at the time: I had very little experience with graph theory, and the movie primarily uses this question and Will Hunting's solving of it as a catalyst for the events of the rest of the movie. Nevertheless, this year I took the math half course Discrete Math Seminar (MADSH) taught by Ms. Lockwood, where we explored graph theory and solved this question together as a class. While this question was written to intentionally appear difficult, with a little background and teamwork, it is actually quite feasible. Try solving this question on your own! The solution is on the next page.



## Solution to the Good Will Hunting Problem:

a) Draw all homeomorphically irreducible trees of size n = 10.



# Rising Through the Thrilling Haze

by Anna Yang '23

In the oldest star, deepest sky,
Laws of nature quietly lie.
Behind the lenses atoms call,
We're the same and different after all.
Straight up into the source we gaze,
Up, up, past the thrilling haze.

Single cells divide and grow,
Then down a one-way road they go.
Microbes, eukaryotes, you and me,
What decides how their fate shall be?
Straight up into the source we gaze,
Up, up, toward the thrilling haze.

Through the tree of life we climb,
Driven by the passing time.
On a field of dust, green was grown
In a world observed but never known.
Straight up into the source we gaze,
Up, up, into the thrilling haze.

Science looks down with peering eyes
And asks the questions deep and wise.
She draws a bridge across the sea
Where all unite to find the key.
Straight up for the truth we chase,
Up, up, through the thrilling haze.

Cover photography by Maya Geyling

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