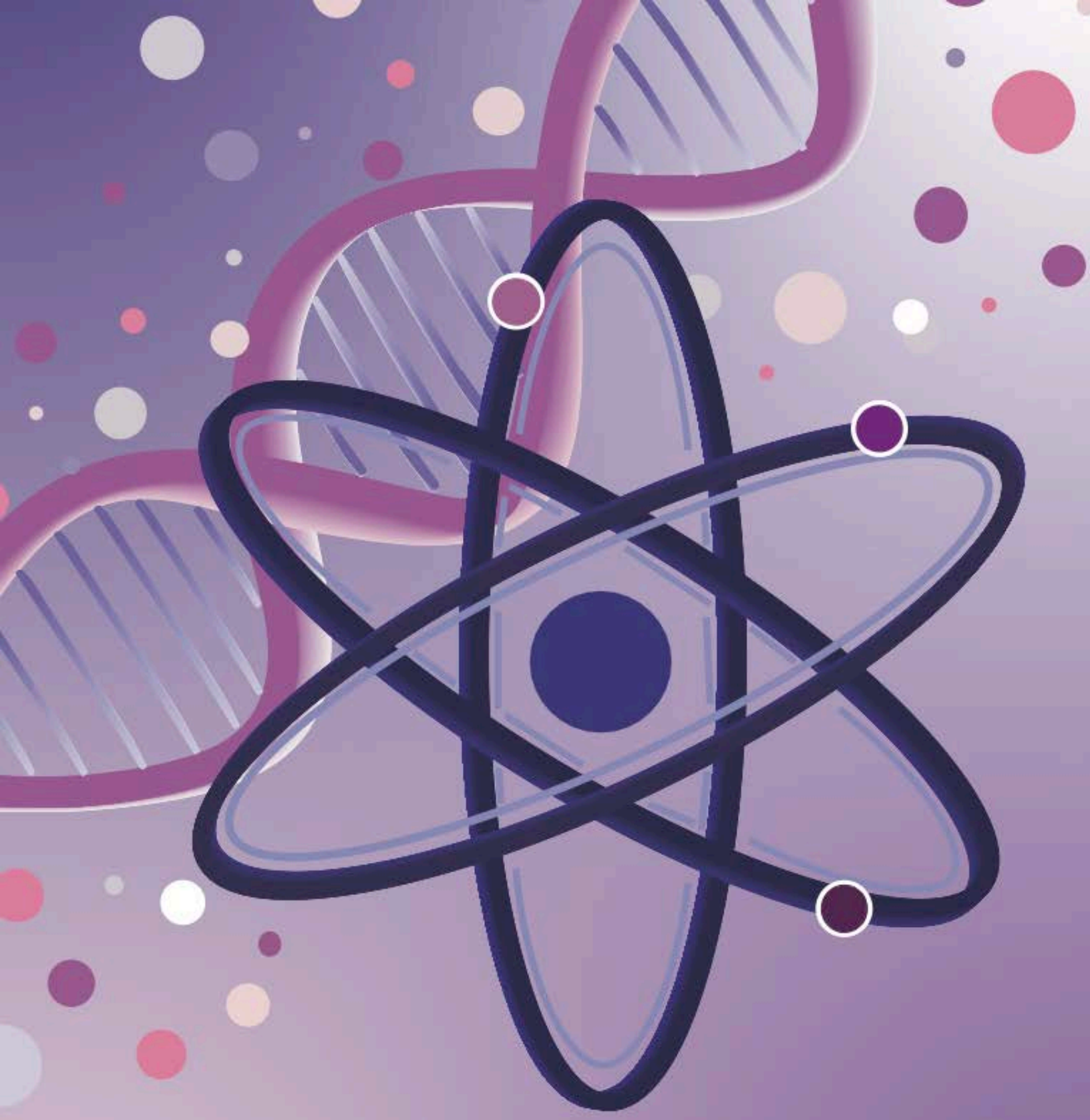


# DHD

VOLUME 1
2025

**DOUBLE HELIX DIGEST AT THE UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN: AN UNDERGRADUATE JOURNAL FOCUSED ON BIOLOGY, CHEMISTRY, AND RELATED FIELDS**

# CONTENTS

It is with immense pride and excitement that we welcome you to the inaugural volume of Double Helix Digest. This publication is the culmination of months of collaboration, curiosity, and creativity from students across disciplines who share one common goal: making science accessible, engaging, and alive.

When we first set out to found DHD, we had no idea what to expect. We only knew that we wanted to share our love of the field. What started as a simple idea quickly grew into something more meaningful than we ever could have imagined. We never anticipated the sheer talent that would emerge from this team. The incredible group of writers, editors, and designers who came together brought not only skill, but a deep sense of purpose to every step of the process. Their passion, creativity, and commitment transformed this journal into a space where science is reimagined.

In an era where scientific literacy is more essential than ever, DHD serves to provide a space for undergraduate voices in science communication. In this issue, you'll find pieces that span fields and formats: from a faculty spotlight on Dr. Shannon Sirk's innovative research, to reviews on protein folding, bird-window collisions, and GLP-1 receptor agonists. Our writers break down urgent topics like antimicrobial resistance and cancer detection, as well as explore the emotional dimensions of dreaming and the behavioral traits that shape personality. In our student research section, you'll see how undergraduates are contributing to real scientific inquiry, including studies on semaglutide and novel treatments for ulcerative colitis.

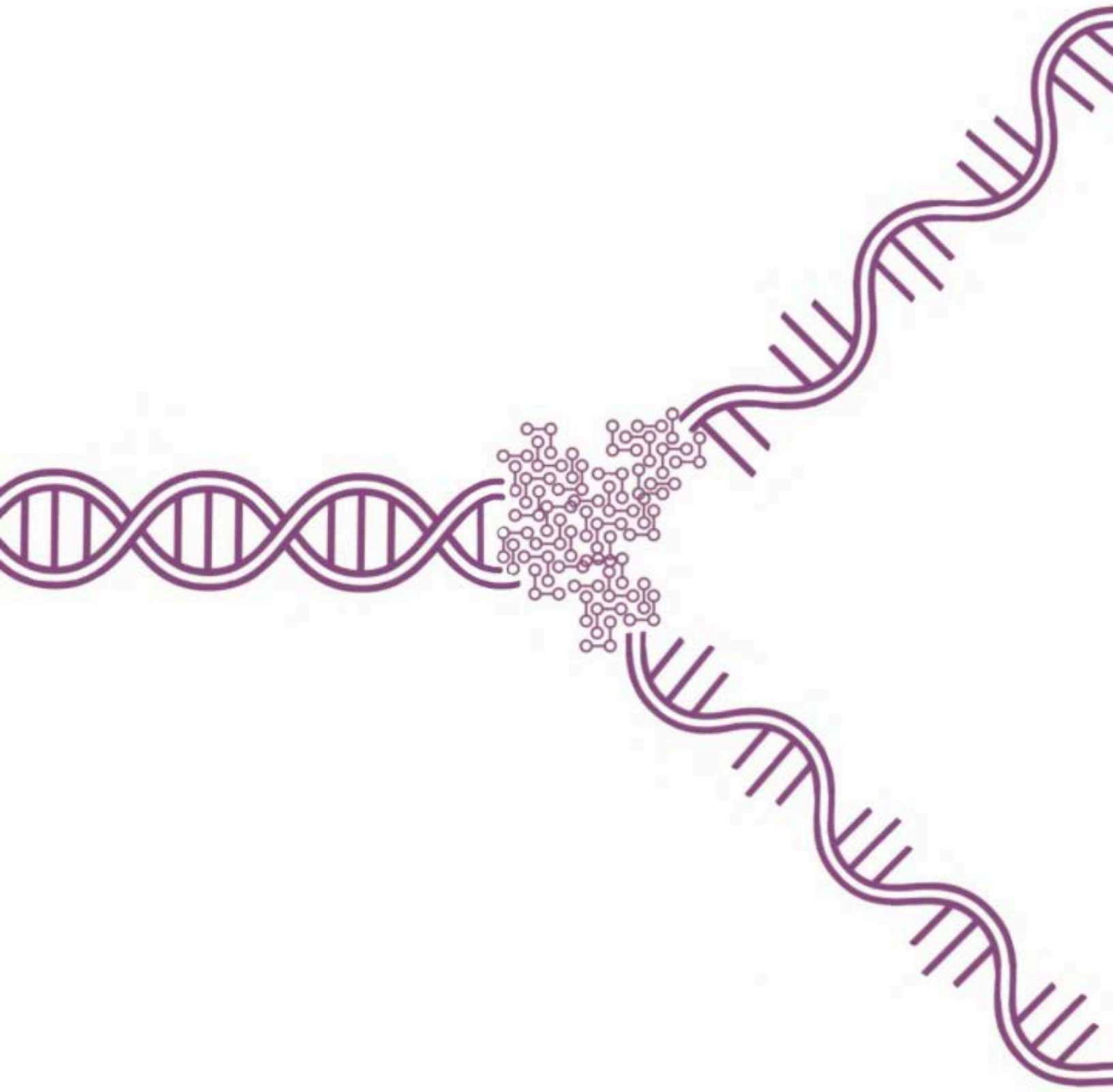
Launching this first volume has been a powerful reminder of what students are capable of. From the first brainstorming sessions to the final rounds of editing, every part of this magazine reflects the initiative, insight, and innovation of our team. Whether you're a seasoned scientist, an aspiring researcher, or simply a curious mind, we hope these pages spark new ideas and challenge you to see science not as a distant discipline, but as an integral thread in the fabric of our daily lives.

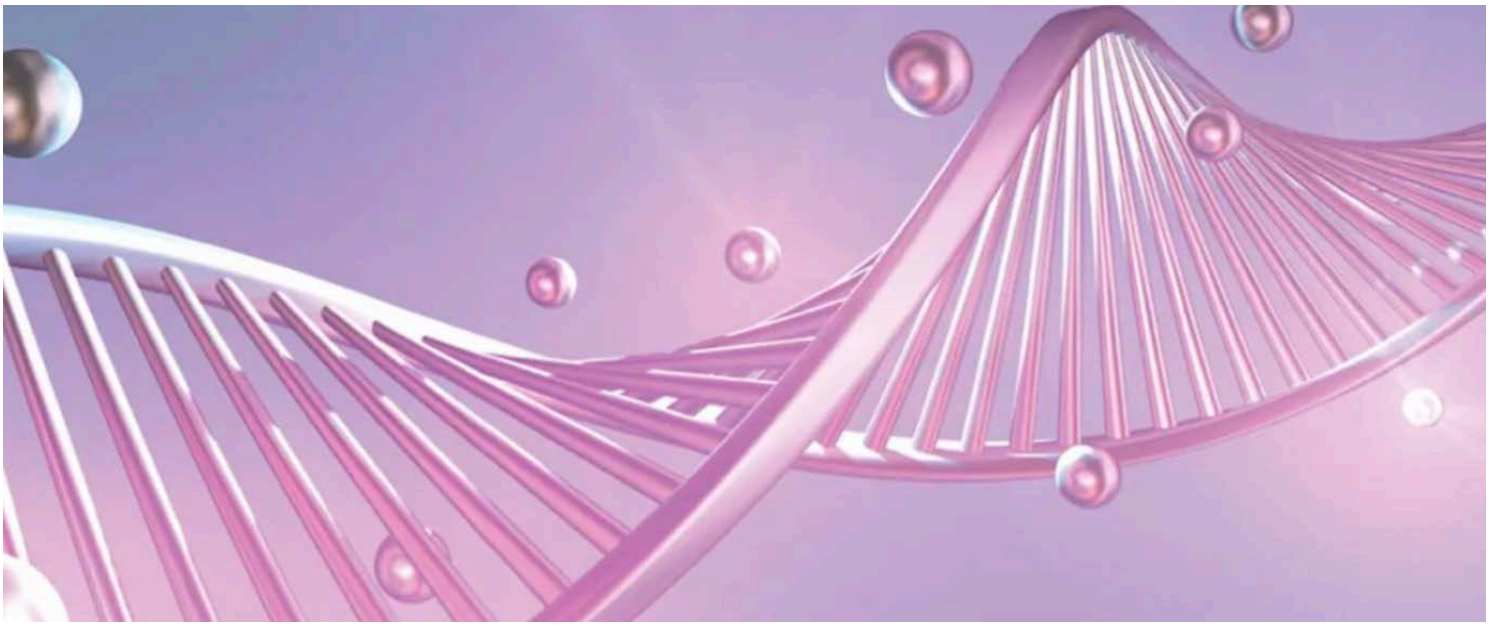
To our team: thank you for trusting in this vision and shaping it into something meaningful. To our readers: thank you for picking up this journal and allowing our voices to reach you.

Here's to many more volumes ahead. Welcome to the first chapter.

Sincerely,
Casey Meskovich & Andrew Hamilton
President & Editor-In-Chief
Double Helix Digest
University of Illinois, Urbana-Champaign

# FACULTY FEATURE





# DR. SHANNON SIRK: BUILDING USEFUL SCIENCE

CASEY MESKOVICH

Magnets of all shapes and sizes dot the walls of Dr. Shannon Sirk's office while her bookshelf overflows with colorful anatomy models– complete with the ever-lingering smell of coffee, the room is nearly as welcoming as she is. By far the most eye-catching addition, however, is a small framed certificate, proudly proclaiming her the "best scientist ever" in scrawling red crayon.

The road to this title started in high school, where her first encounter with protein synthesis sparked what would become a lifelong passion. As she learned how ribosomes translate genetic code into functional proteins, she found herself captivated by the field of molecular and cellular biology. This growing interest led her to Occidental College, where she immersed herself in undergraduate biology studies, eagerly absorbing everything from biochemistry to genetics.

Though Dr. Sirk planned on further fostering this interest in graduate school, she decided to first pursue hands-on experience in the field. Her first professional position at City of Hope's gene therapy program proved transformative. Here, she began to appreciate how fundamental biological principles could be harnessed to develop therapeutic solutions. Seeking even broader exposure, she then undertook a unique dual role, splitting her time between the Jet Propulsion Laboratory (JPL) and Children's Hospital Los Angeles.

Armed with hands-on experience from her time in gene therapy and interdisciplinary research, Dr. Sirk embarked on her doctoral studies at the University of California, Los Angeles (UCLA). Her graduate work not only deepened her technical skills but also sharpened her ability to frame scientific questions, laying the groundwork for her future as an independent investigator. At Scripps Research, she discovered the creative potential of protein engineering, a field that married her love of molecular mechanisms with tangible design applications.

Her subsequent position at Stanford University, however, presented an entirely different challenge. As the sole biologist in a chemistry-focused lab, she navigated unfamiliar methodologies and often worked in isolation. While the project yielded just one publication, the experience proved unexpectedly valuable. She credits this experience with teaching her to be self-sufficient in research. “You can be good at what other people tell you to do,” she says, “but learning to plan a project yourself—that’s the hard part.”

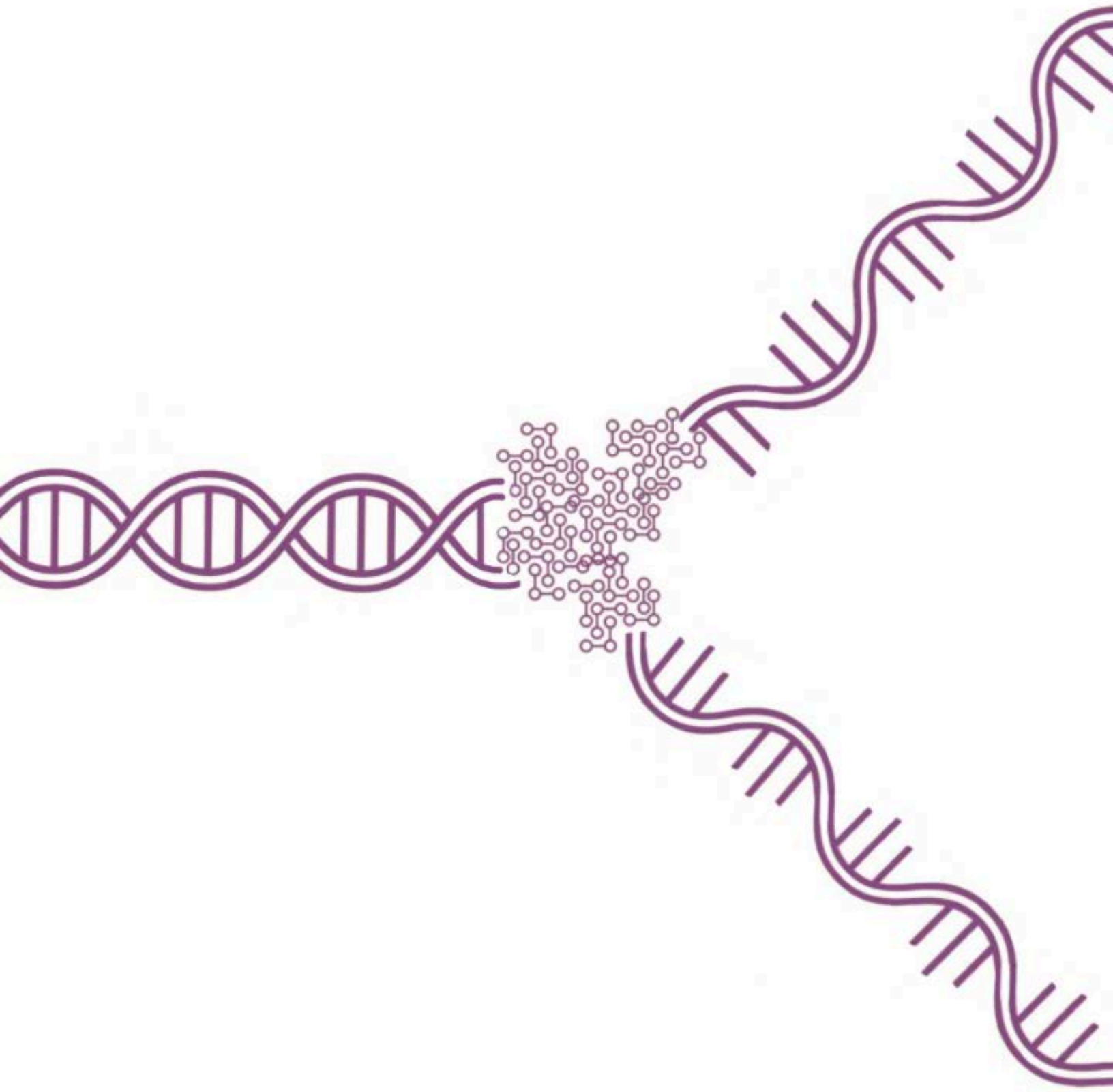
Now leading the Sirk Group at the University of Illinois Urbana-Champaign, Dr. Sirk channels her hard-won independence into a singular goal. "I just want to do stuff that’s useful," she says—and her lab does just that. Their research focuses on developing next-generation biotherapeutics by engineering both the therapeutic molecules and the living systems that deliver them. This includes designing microbial “living therapeutics” that can safely colonize the human body and produce treatment molecules on-site, as well as engineering the protein-based therapeutics themselves—often compact antibody fragments optimized for bacterial production, stability, and in vivo performance. By combining synthetic biology, protein engineering, and immunology, the Sirk Group is pushing toward therapies that are smarter, more adaptable, and more precise—solutions that could reshape disease treatment in humans, animals, and even the environment.

Dr. Sirk stresses that there is no single solution to the kinds of problems her lab takes on, and emphasizes the importance of being deeply knowledgeable in the field—not only to recognize which problems are worth solving, but to design creative, effective solutions. This mindset is something she encourages in her students as well. Her advice to undergraduates is clear: be patient, and commit to the process. “You don’t get to a place where you feel like you can do the job until you’re already doing it,” she says. Research isn’t just about knowing the right answers—it’s about learning to ask better questions, and embracing the slow, often nonlinear work of discovery. She encourages students to keep reading, keep showing up in the lab, and to treat research not just as coursework, but as a craft—something that improves with practice. “You don’t always love it, and it’s not always fun,” she adds, “but you *can* always get better—at running a gel, reading a paper, giving a presentation.”

Looking back, Dr. Sirk is the first to admit that her path wasn’t linear—and certainly not conventional. Each turn in her journey added a new lens through which to view complex problems. Though trained as a molecular biologist, her work has intersected with optical engineers, cancer researchers, and immunologists, giving her the ability to speak across disciplines and think in many dimensions at once. That wide-ranging experience shapes not only how she does science, but how she mentors others to find their own paths through it. In that sense, the red crayon certificate in her office—the one proclaiming her the “best scientist ever”—feels like a reflection of the kind of scientist she’s become: not just skilled, but open-minded, adaptable and endlessly curious.

*Image credits: “DNA Not Necessarily Your Destiny: The Role of Epigenetics in Pharmacy.” The Pharmaceutical Journal, 6 Sept. 2017, Sirk, Shannon. “Shannon Sirk.” School of Molecular & Cellular Biology, University of Illinois Urbana-Champaign*

# REVIEW ARTICLES



# FACTORS AFFECTING MIGRATORY BIRD-WINDOW COLLISIONS AND EFFECTIVE MITIGATION STRATEGIES

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**ABSTRACT**
Bird-window collisions pose a major threat to bird populations, killing up to a billion birds annually in the United States alone. Migratory birds, many of which use the stars and Earth's magnetic field to navigate during the night, are particularly vulnerable to collisions since anthropogenic factors such as light pollution can disrupt their ability to navigate. Factors such as species behavior, surrounding vegetation, and window design have been found to influence collision rates. Research has also identified several effective mitigation strategies, including bird-safe window treatments, reduced artificial light at night, and public education. Addressing the threat of window collisions is crucial to protect avian biodiversity worldwide and preserve the ecological benefits provided by birds.

## INTRODUCTION

Volunteers from the Chicago Field Museum were met with a gruesome sight after the night of October 4th, 2023, one of the largest migration nights of the season: approximately a thousand dead birds were scattered by the windows of Chicago's McCormick Place. The building had already been known to be problematic to birds due to its extensive glass facade, but a particularly significant migration night and poor weather conditions led to the largest number of deaths ever recorded at the building. Since then, bird-safe film has been installed in all the building's windows after a $1.2 million project, leading to a 95% reduction in collisions according to McCormick Place (2025).

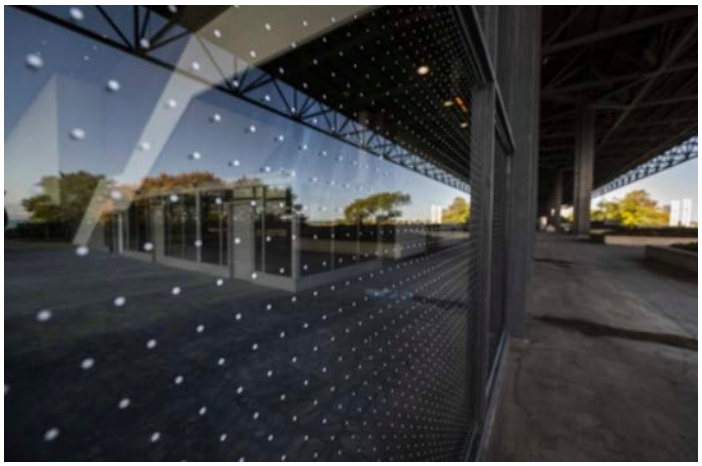

Figure 1: The new bird-safe film installed at McCormick Place, Chicago (Groleau 2024)

The tragedy at McCormick Place is just one example of how bird-window collisions pose an extreme threat to the stability of bird populations, with such strikes estimated to lead to up to a billion bird deaths in the US alone each year (Loss et al. 2014). This firmly places window collisions as the second leading cause of death in the US for birds, only surpassed by cats. Window collisions often occur because birds are unable to perceive windows as barriers due to their transparent properties. Furthermore, windows can reflect surrounding habitat, causing the bird to mistake a window as vegetation that they then fly towards (Klem 1989). While window collisions pose a threat to all birds, migratory birds are especially at risk due to their tendency to migrate at night. Many species of birds use the stars to help navigate at night, meaning that light pollution from cities can disorient and attract them, consequently increasing the risk of a window collision as birds are drawn to more urbanized environments. A significant number of temperate birds migrate during the fall and spring seasons to follow seasonal resource availability among other benefits. Approximately 70% of terrestrial birds that regularly occur in North America are considered to be migratory (Horton et al. 2019). This widespread migratory behavior means that many species in North America are vulnerable to window collisions, putting their populations in greater risk of decline.

Research has shown that around 57% of North American bird species have seen population declines since 1970, corresponding to a decrease of 3 billion birds. This is a loss of 29% of 1970 bird abundance levels (Rosenberg et al. 2019). Such staggering losses underline the need to focus on the primary threats to birds, such as window collisions, and identify effective mitigation strategies to ensure the long-term stability of bird populations. Birds provide a range of ecosystem services, including pollination, seed dispersal, and pest control, so it is important to preserve these populations in order to prevent any associated losses in ecological stability in the process (Whelan, Wenny, & Marquis 2008).

## OVERVIEW OF BIRD MIGRATION BIOLOGY

Migration plays an important role in many avian species since the change of seasons leads to fluctuations in the availability of resources in their habitats. Birds that most commonly migrate include songbirds (order Passeriformes), waterfowl (order Anseriformes), and shorebirds (order Charadriiformes). These birds aim to move to areas with increasing resources while leaving areas with decreasing resources. Approximately 80% of migratory birds in North America choose to migrate in the night for reasons that are not fully understood yet (Horton et al. 2019). However, some evidence has been found to support the hypothesis that nocturnal behavior helps animals face reduced competition and fewer predators (Wcislo et al. 2004).

A variety of methods are used by birds to navigate the skies during migration, many of which can be disrupted by anthropogenic factors. Scientists in 1977 performed an experiment that observed bird flight

patterns of the garden warbler (*Sylvia borin*) and the European robin (*Erithacus rubecula*) under various conditions, finding that the presence of stars had a positive effect on the ability of these birds to maintain a flight direction, and that robins were unable to choose a meaningful direction under the absence of a magnetic field (Wiltschko 1978). Recent studies have confirmed and added on to these findings over the decades. Foster et al. describe in their review article that many night-migrating birds identify the star-filled sky's center of rotation as their reference of orientation (2018). It was also discovered that space weather can disrupt nocturnal bird migration, given that birds rely on Earth's magnetic field. There was a 9 to 17% decrease in migration intensity observed during severe space weather events such as bursts of solar energy, which affect Earth's magnetic field (Gulson-Castillo et al. 2023). The methods birds use to navigate during migration are still not fully understood at the moment, with more research currently being conducted on the topic. Understanding the mechanisms that underlie migration helps with recognizing the potential effects of light pollution on migration and how it factors into bird-window collisions.

Migratory birds are particularly vulnerable to population declines due to a variety of factors, which is only exacerbated by their vulnerability to window collisions. Migratory birds move between various geographical regions and can thus be affected by factors in different parts of the world. Scientists from the National Audubon Society have predicted that two thirds of bird species in North America are vulnerable to extinction as a result of climate change. Climate change can lead to a potential mismatch between the emergence of insects and bird migration, posing risks for these populations (Bateman et al. 2020). Moreover, nearly all the bird species at high risk for both low-rise and high-rise birds were found to be migratory since they traverse longer distances over the course of the year, encountering more building types and total buildings (Loss et al. 2014). As scientific research continues to unravel the secrets behind bird migration, there is an urgent need to address the primary conservation challenges migratory birds face due to anthropogenic activity such as bird-window collisions, climate change, and habitat loss. As urbanization accelerates, these dangers will only intensify, putting migratory bird populations at increasing risk.

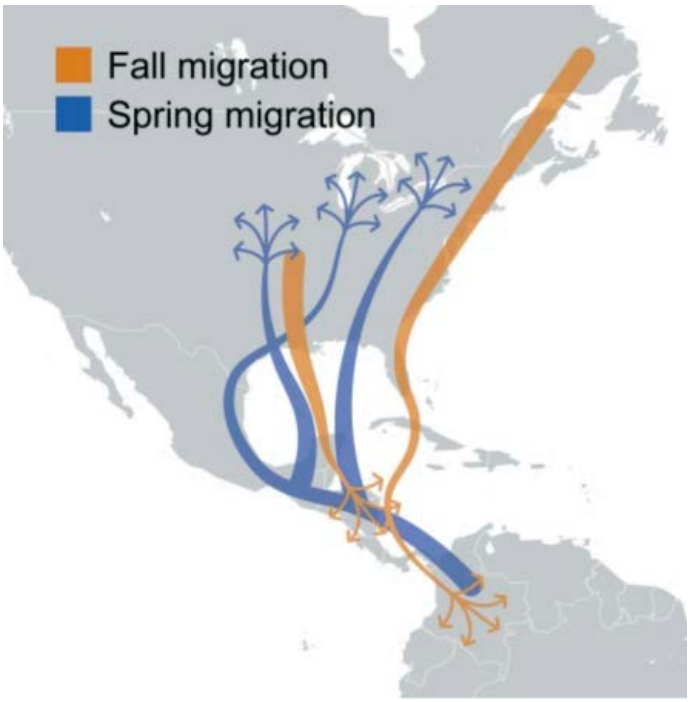


Figure 2: A map depicting the migration routes of birds in North America (U.S. Fish & Wildlife Service 2023)

**BIRD-WINDOW COLLISION FACTORS**

There are a multitude of factors affecting the rate at which bird-window collisions occur. Understanding these can help pinpoint which mitigation strategies may be the most effective. For one, certain species are more vulnerable due to their behavior and flight patterns. In North America, species of warblers, thrushes, vireos, and sparrows tend to be some of the most commonly found victims, all of which exhibit high levels of migration. The cause of these observations is not fully understood, but it is known that these species tend to fly through densely vegetated areas and are heavily guided by light in their flight, so sources of artificial light can be more disruptive. Other vulnerable species, such as ovenbirds and various thrush species, spend a significant amount of their time on the ground, thus increasing proximity to windows. Furthermore, research suggests that species that primarily migrate at night are more susceptible to window collisions compared to diurnal (daytime) migrants, potentially due to being attracted towards urban areas as a consequence of light pollution (Ogden 1996; Nichols et al. 2018).

Moreover, researchers have found that window proximity to surrounding vegetation and bird feeders plays a significant role in collision rates. A team of researchers in Pennsylvania manipulated the distance of a bird feeder to a window between 1, 5, and 10 meters. Their results showed an increase in window fatalities with a distance of 5 and 10 meters, with a distance of 1 meter leading to no fatalities (Klem et al. 2004). These findings are potentially explained by the fact that birds flying from feeders close to windows do not have enough time to accelerate to a point that a window collision becomes fatal. Another study, taking place in Xalapa, Mexico, found a significant positive relationship between the amount of surrounding vegetation area of a building and the rate of bird-window collisions (Gómez-Martínez et al. 2019). Vegetation is often reflected in windows, confusing birds as they perceive the reflection as actual vegetation that they try to fly to. More vegetation increases the chance this occurs. In summary, greater vegetation near windows increases collisions, while shorter distances between feeders and windows reduce fatal impacts.

Window design, such as the type of window and its angle to the ground is another factor that plays a role in bird-window collisions. Window panels facing the ground have a reduced collision rate, attributed to reduced reflections of surrounding vegetation and the sky (Klem et al. 2004). The size of glass panes had a significant effect on the number of bird collisions with windows. In fact, dividing large glass panes into smaller panels has been found to lead to lower collision rates (Kahle, Flannery, & Dumbacher 2016). Additionally, glass panels that are more reflective in nature increase the chance of collisions, once again due to amplifying reflections of the surrounding environment, drawing birds towards what they perceive as natural habitats (Klem & Saenger 2013).

Perhaps the most significant factor in bird-window collisions is light pollution. Artificial light at night plays an especially important role in bird fatalities. Studies have shown that urban light installations have led to dramatic alterations of nocturnal bird migration. For instance, the beams of the National September 11 Memorial & Museum's "Tribute in Light" in New York City were found to alter the behavior of around 1.1 million birds over seven days, observed over seven years. Observed behavioral changes included decreased flight speeds, circular flight paths, and frequent vocalizations. Bird densities around the area were also 20 times higher than the baseline (Van Doren et al. 2017). This gives credence to the idea that light pollution can increase vulnerability to window collisions, owing to the high number of birds attracted to urbanized areas. Significant fatalities can occur when this is combined with poor weather, like what happened at McCormick Place. Specifically, unfavorable winds and weather that impedes visibility increases the rates of bird-building collisions during migration seasons (Chen et al. 2024). A study taking place in Minneapolis found that the area of glass emitting artificial light at night was the most important factor when it came to collisions, being a better predictor than the glass area, glass percentage, and the maximum and average sizes of glass panes (Lao et al. 2020). Beyond just the lighting area, the proportion of windows lighted was an important predictor. Halving the number of lighted windows was estimated to decrease the number of collisions by a magnitude of 11 in the spring and 6 in the fall (Van Doren et al. 2021). This is a remarkable improvement, underlying the need to turn off lights whenever not in use, especially during the night when nocturnal migrants are active.

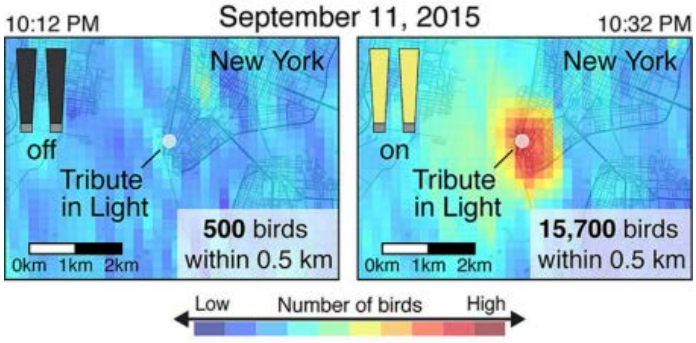


Figure 3: Bird densities at the National September 11 Memorial & Museum "Tribute in Light" display in New York City (Van Doren et al. 2017)

## MITIGATION STRATEGIES

Despite the staggering losses caused by bird-window collisions, the issue of bird-window collisions is certainly remediable and researchers have identified several mitigation

strategies that can counter the various factors causing collisions.

Modifying windows to be bird-friendly is one of the most effective mitigation strategies. In the past, suggestions have been made to apply decals and hang objects such as cords in front of glass to prevent collisions, but these were based on educated guesswork rather than empirical evidence. However, recent studies have since shown that these strategies are indeed effective. For instance, a team of researchers at the University of Utah found that applying Feather Friendly® bird deterrent markers to collision-prone windows led to a statistically significant decline in bird-window collisions in contrast to the untreated windows that formed the control group (Brown, Santos, & Ocampo-Peñuela 2021). Another study conducted in Pennsylvania found that stripe and grid patterns of window coverings that are UV-reflecting or UV-absorbing can warn birds of glass while not obstructing the view for humans, since birds are able to see UV wavelengths. The study also found that one-way films that make the outer surface of a window opaque or translucent and windows covered with decals 5-10 cm apart were extremely effective at preventing bird strikes (Klem 2009). Bird-friendly artwork was incorporated at the University of British Columbia, leading to a significant reduction in collisions while also being aesthetically appealing (Crews 2022). Homeowners and conservation practitioners both showed positive views towards bird collision management measures. This suggests that great strides can be made towards increasing the prevalence of bird-friendly windows, underlying the need for education and awareness programs to gain public support for bird-friendly windows (Riggs, Joshi, & Loss 2022).

Even “green” and “environmentally-focused” developments widely use glass panes in their designs, failing to take into account the risk of collisions for birds. This is one of the drawbacks of Leadership in Energy and Environmental Design (LEED) certifications, as LEED-certified buildings tend to have a large portion of its facade being composed of windows, posing a greater danger to birds. Furthermore, the certification promotes increased vegetation around buildings, which has been shown to increase bird-window collisions. These lead to unfortunate side effects despite the intention of being environmentally-friendly (Ocampo-Peñuela et al. 2016). Being environmentally-conscious and bird-friendly, however, are not mutually exclusive. There are assuredly solutions that can be incorporated into the LEED certification process, such as bird-safe window glazings that align with LEED guidelines. Further research is required to determine whether there can be a compromise between surrounding vegetation for LEED certification and bird-friendly building design (Tews 2022).

Figure 4: An example of bird-safe window decals at the University of British Columbia Botanical Garden Pavillion (Crews 2022)

Certain LEED recommendations, however, do benefit bird safety, such as the recommendation to turn off lights when dark out to save energy. Studies have shown that turning off lights, especially in the night, can lead to significant reductions in bird-window collisions. Data taken from the Bird Friendly Building Program (BFB), as part of the Fatal Light Awareness Program (FLAP) in Toronto, Canada revealed that decreases in light emissions from a building were positively correlated with decreases in bird fatalities and injured birds found as a result of window collisions. For this reason, Lights Out programs are crucial to prevent bird fatalities during the busy migration seasons. These programs have now spread to over 30 cities in North America, and continue to gain popularity (Ogden 1996).

These efforts have spread to college campuses as well, such as the Illini Lights Out program, which aims to engage the community to turn off lights around campus for the weekend to conserve energy and reduce artificial light at night. These programs can be made even more effective by identifying heavy migration nights through the use of bird migration forecasting tools, such as BirdCast. Aside from preventing bird deaths, Lights Out programs have the added benefit of reducing power consumption and leading to a reduction in operating costs, thus being a win-win solution for all those involved.

Engaging and educating the public plays an important role as well. Citizen science campaigns are crucial to collect data in order to determine the most effective methods of prevention. Citizen science refers to research conducted with the participation of the general public through volunteering. These campaigns allow for research to be collected in greater numbers than otherwise possible due to limitations. Much of the research previously discussed was conducted with the help of the general public. Loss et al. note how the data provided by citizen science programs have played an important role in advancing bird-window collision research. Additionally, citizens have advocated for bird-friendly building policies and have helped raise awareness of the issue, which has helped increase funding towards research focused on the topic (2023). Numerous successful campaigns have been conducted around the world, including but not limited to Lights Out Texas and the China Anti-Bird Window Collision Action Alliance. Efforts extend to the University of Illinois Urbana-Champaign as well, with members of the UIUC Bird Strike Survey volunteering to record bird-window collisions around campus during the fall and spring migration seasons. It is especially important to raise awareness on a global scale, as the majority of the research conducted on bird-window collisions has been conducted in the Northern Hemisphere, especially in the USA and Canada, despite the diversity of bird species found in the tropics. One of the issues with mitigation is the lack of scientific knowledge regarding bird-window collisions in tropical countries, which can make it difficult to extrapolate findings specific to temperate regions (Basilio, Moreno, & Piratelli 2020). Citizen science programs in these underrepresented regions of the world can help researchers gain a more comprehensive understanding of bird-window collisions. Ultimately, increased awareness of bird-window collisions is crucial to gain support to put into place the various strategies for mitigation, especially since attention from major conservation organizations and the government has been quite limited thus far.

**CONCLUSION**

Bird-window collisions are a significant threat to the populations of many bird species, with a wide range of consequences. Understanding the various factors that play a role in bird-window collisions (such as artificial light pollution, window proximity to vegetation, and window reflectivity) is critical when it comes to devising effective mitigation strategies. Measures such as limiting artificial light at night and modifying windows with bird-friendly designs can help significantly reduce bird collisions. Moreover, educating the public through citizen science campaigns can lead to increased attention and funding for conservation efforts and allow for better data collection to gain a more comprehensive understanding of bird-window collisions.

However, there are limitations to current research and mitigation efforts. Most studies focus on urban areas in North America and Europe, leaving gaps in understanding bird-window collisions in other regions, particularly in the Global South. Furthermore, while certain mitigation strategies have proven to be effective in controlled settings, large-scale implementation of these ideas remains a challenge due to economic and logistical reasons. Future research needs to be done on the effectiveness of mitigation techniques across a diverse range of environments, long-term population-level impacts, and species-specific vulnerabilities.

If said measures are not adopted and implemented on a wider scale, then threats to birds from window collisions are poised to increase due to the urbanization taking place across the world. Avian fauna contribute a wide variety of ecosystem services and other recreational benefits, so it is pivotal to

address the threat of bird-window collisions to preserve the role birds play in the lives of humans and the overall biosphere.

**REFERENCES**


1. A commitment to bird conservation at McCormick Place. McCormick Place. (2025, February 25).

2. Basilio, L. G., Moreno, D. J., & Piratelli, A. J. (2020). Main causes of bird-window collisions: A Review. Anais Da Academia Brasileira de Ciências, 92(1).

3. Bateman, B. L., Wilsey, C., Taylor, L., Wu, J., LeBaron, G. S., & Langham, G. (2020). North American birds require mitigation and adaptation to reduce vulnerability to climate change. Conservation Science and Practice, 2(8).

4. Brown BB, Santos S, Ocampo-Peñuela N. (2021). Bird-window collisions: Mitigation efficacy and risk factors across two years. PeerJ 9:e11867.

5. Chen, K., Kross, S. M., Parkins, K., Seewagen, C., Farnsworth, A., & Van Doren, B. M. (2024). Heavy migration traffic and bad weather are a dangerous combination: Bird collisions in New York City. Journal of Applied Ecology, 61(4), 784–796.

6. Crews, C. (2022). Reducing bird-window collisions at a botanical garden : The effect of bird-friendly artwork and dirty windows [R].

7. Gómez-Martínez, M.A., Klem, D., Rojas-Soto, O., González-García, F., & MacGregor-Fors, I. (2019). Window strikes: bird collisions in a Neotropical green city. Urban Ecosystems 22, 699–708.

8. Groleau, P. (2024). McCormick Place in Chicago Treats Windows to Prevent Bird Collisions. [Image].

9. Gulson-Castillo, E. R., Van Doren, B. M., Bui, M. X., Horton, K. G., Li, J., Moldwin, M. B., Shedden, K., Welling, D. T., & Winger, B. M. (2023). Space weather disrupts nocturnal bird migration. Proceedings of the National Academy of Sciences, 120(42).

10. Horton, K. G., Nilsson, C., Van Doren, B. M., La Sorte, F. A., Dokter, A. M., & Farnsworth, A. (2019). Bright lights in the big cities: Migratory birds' exposure to artificial light. Frontiers in Ecology and the Environment, 17(4), 209–214.

11. Foster, J. J., Smolka, J., Nilsson, D.-E., & Dacke, M. (2018). How animals follow the stars. Proceedings of the Royal Society B: Biological Sciences, 285(1871).

12. Kahle, L. Q., Flannery, M. E., & Dumbacher, J. P. (2016). Bird-window collisions at a west-coast urban park museum: Analyses of bird biology and window attributes from Golden Gate Park, San Francisco. PLOS ONE, 11(1).

13. Klem, D., & Saenger, P. G. (2013). Evaluating the effectiveness of select visual signals to prevent bird-window collisions. The Wilson Journal of Ornithology, 125(2), 406–411.

14. Klem, D., Keck, D. C., Marty, K. L., Ball, A. J. M., Niciu, E. E., & Platt, C. T. (2004). Effects of window angling, feeder placement, and scavengers on avian mortality at plate glass. The Wilson Bulletin, 116(1), 69-73.

15. Klem, Jr, Daniel. (1989). Bird: Window Collisions. The Wilson Bulletin, 101(4), 606–620.

16. Klem, Jr, Daniel. (2009). "Preventing Bird–Window Collisions," The Wilson Journal of Ornithology 121(2), 314-321.

17. Lao, S., Robertson, B. A., Anderson, A. W., Blair, R. B., Eckles, J. W., Turner, R. J., & Loss, S. R. (2020). The influence of artificial light at night and polarized light on bird-building collisions. Biological Conservation, 241.

18. Loss, S. R., Li, B. V., Horn, L. C., Mesure, M. R., Zhu, L., Brys, T. G., Dokter, A. M., Elmore, J. A.,Gibbons, R. E., Homayoun, T. Z., Horton, K. G., Inglet, P., Jones, B. J., Keys, T., Lao, S., Loss, S. S., Parkins, K. L., Prestridge, H. L., Riggs, G. J., ... Farnsworth, A. (2023). Citizen science to address the global issue of bird–window collisions. Frontiers in Ecology and the Environment, 21(9), 418–427.

19. Loss, S. R., Will, T., Loss, S. S., & Marra, P. P. (2014). Bird–building collisions in the United States: Estimates of annual mortality and species vulnerability. The Condor, 116(1), 8–23.

20. Nichols, K. S., Homayoun, T., Eckles, J., & Blair, R. B. (2018). Bird-building collision risk: An assessment of the collision risk of birds with buildings by phylogeny and behavior using two citizen-science datasets. PLOS ONE, 13(8).

21. Ocampo-Peñuela, N., Winton, R. S., Wu, C. J., Zambello, E., Wittig, T. W., & Cagle, N. L. (2016). Patterns of bird-window collisions inform mitigation on a university campus. PeerJ, 4.

22. Ogden, L. J. E., World Wildlife Fund Canada, & Fatal Light Awareness Program. (1996). Collision course: the hazards of lighted structures and windows to migrating birds. World Wildlife Fund Canada.

23. Riggs G. J, Joshi O, & Loss S. R. (2022). Stakeholder perceptions of bird-window collisions. PLoS ONE 17(2): e0263447.

24. Rosenberg, K. V., Dokter, A. M., Blancher, P. J., Sauer, J. R., Smith, A. C., Smith, P. A., Stanton, J.C., Panjabi, A., Helft, L., Parr, M., & Marra, P. P. (2019). Decline of the North American avifauna. Science, 366(6461), 120–124.

25. Tews, A. (2022). LEED certified buildings and bird-friendly window design. UF Journal of Undergraduate Research, 24.

26. U.S. Fish & Wildlife Service. (2023). Migration Map [Image].

27. Van Doren, B. M., Horton, K. G., Dokter, A. M., Klinck, H., Elbin, S. B., & Farnsworth, A. (2017). High-intensity urban light installation dramatically alters nocturnal bird migration. Proceedings of the National Academy of Sciences, 114(42), 11175–11180.

28. Van Doren, B. M., Willard, D. E., Hennen, M., Horton, K. G., Stuber, E. F., Sheldon, D., Sivakumar, A.H., Wang, J., Farnsworth, A., & Winger, B. M. (2021). Drivers of Fatal Bird Collisions in an urban center. Proceedings of the National Academy of Sciences, 118(24).

29. Wcislo W. T., Arneson L., Roesch K., Gonzalez V., Smith A., & Fernández H. 2004 The evolution of nocturnal behaviour in sweat bees, Megalopta genalis and M. ecuadoria (Hymenoptera: Halictidae): an escape from competitors and enemies? Biol. J. Linn. Soc. 83, 377–387.

30. Whelan C. J., Wenny D. G., & Marquis R. J. Ecosystem services provided by birds. Ann N Y Acad Sci. 2008;1134:25-60.

31. Wiltschko, R., & Wiltschko, W. (1978). Relative Importance of Stars and the Magnetic Field for the Accuracy of Orientation in Night-Migrating Birds. Oikos, 30(2), 195–206.

# HOW RIBOSOMES SHAPE PROTEIN FOLDING

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**ABSTRACT**
Protein folding is a fundamental biological process essential to cellular function, yet its mechanisms (particularly those occurring during synthesis) remain incompletely understood. Traditionally viewed as a post-translational event, mounting evidence now reveals that protein folding often begins co-translationally, with the ribosome playing a direct and active role. This review examines how ribosomes contribute to co-translational folding by providing a confined, highly regulated environment within the exit tunnel that shapes early folding events, guides hydrophobic collapse, and coordinates folding with translation speed. Structural and thermodynamic studies reveal that the ribosome minimizes entropic penalties and promotes stable intermediate states, thus enhancing folding efficiency and fidelity. Furthermore, ribosome-associated chaperones assist in preventing misfolding and aggregation. These insights not only reshape our understanding of the central dogma of protein science but also have profound implications for disease research, including cancer and neurodegenerative disorders, and offer new targets for therapeutic intervention aimed at regulating proteostasis during translation.

## THE PROBLEM

Proper protein folding is crucial for cellular function, and errors in this process are linked to a variety of debilitating diseases, including neurodegenerative disorders like Alzheimer's and certain types of cancer. Despite this, the process by which proteins fold into their functional structures remains one of the most complex and poorly understood aspects of cellular biology.

Although significant advances have been made in predicting the final three-dimensional shapes of proteins from their amino acid sequences, the mechanism by which these shapes form during protein synthesis remains largely elusive. Once unfolded, many proteins fail to refold easily, tend to misfold and aggregate, and require the help of chaperones to attain their correct structure (Samatova, Komar, & Rodnina, 2023). This directly negates the central dogma of protein science, which is that the amino acid sequence of the protein defines its three-dimensional structure (Nature Publishing Group, 2024). This indicates that most proteins do not fold into their active forms freely in solution; instead, their folding begins while they are still being synthesized by the ribosome—the cellular machinery responsible for protein translation (Samatova et al., 2023).

## AN INTRODUCTION TO RIBOSOMES AND PROTEIN SYNTHESIS

To understand how ribosomes influence protein folding, it's important to first grasp their role in protein synthesis. A ribosome is a cellular organelle found in the cytoplasm or bound to the endoplasmic reticulum and is primarily responsible for protein synthesis. Because of their essential role, cells contain a large number of ribosomes. The exact number varies depending on the cell type and its level of protein synthesis activity (Nature Publishing Group). For instance, a single mammalian cell can house up to ten million ribosomes (British Society for Cell Biology).

A ribosome is made up of two subunits, deemed "large" and "small." The large subunit is about twice the size of the small subunit and functions mainly as a catalyst, whereas the small subunit is typically a decoder (British Society for Cell Biology). The small subunit binds to messenger RNA (mRNA) and reads its nucleotide sequence, ensuring that the information is translated correctly. Once the small subunit attaches to the mRNA, the large subunit then locks onto the small subunit, forming a functional ribosome complex. This assembly creates a scaffold where the process of translation takes place (Genome.Gov).

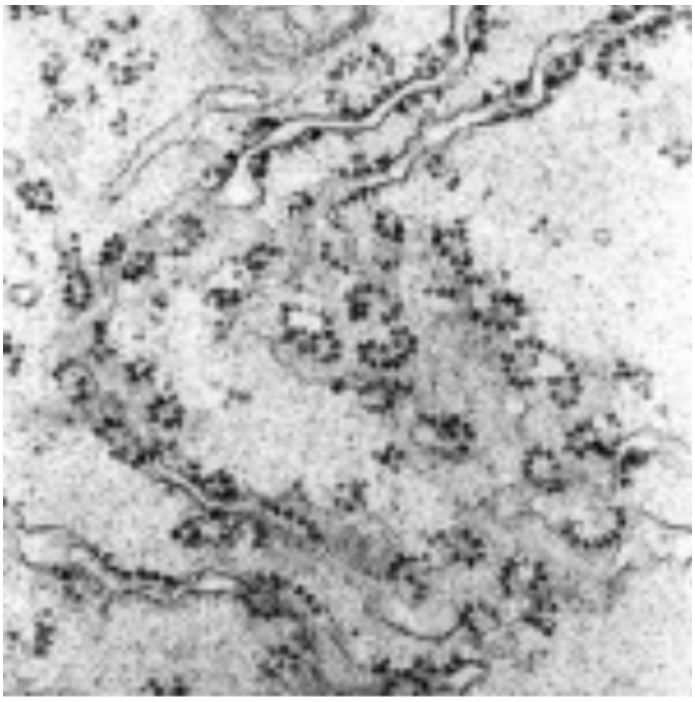


Figure 1: An electron microscope image showing part of the rough endoplasmic reticulum in a plant root cell from maize, in which the dark spots are ribosomes (British Society for Cell Biology)

Translation begins when the ribosome encounters the start codon of an mRNA strand. As the ribosome moves along the mRNA, transfer RNA (tRNA) molecules enter the ribosome, bringing along specific amino acids. The large subunit catalyzes the formation of peptide bonds between the amino acids, elongating the polypeptide chain. The ribosome continues reading the mRNA, adding one amino acid at a time until it reaches a stop codon, at which point translation halts, and the newly synthesized polypeptide is released (British Society for Cell Biology).

## THE ROLE OF RIBOSOMES IN CO-TRANSLATIONAL FOLDING

While ribosomes are most commonly known for their primary function in protein synthesis, recent studies have shown that they also play a crucial and active role in the folding of nascent

proteins (proteins that have been synthesized, but have not yet folded into their final shape). This process, known as co-translational folding, refers to the folding of a protein as it is being synthesized by the ribosome. Unlike post-translational folding, where proteins fold after they are completely synthesized, co-translational folding happens simultaneously with translation. This allows the nascent polypeptide chain to begin adopting its functional three-dimensional structure while it is still attached to the ribosome (Samatova et al., 2023).

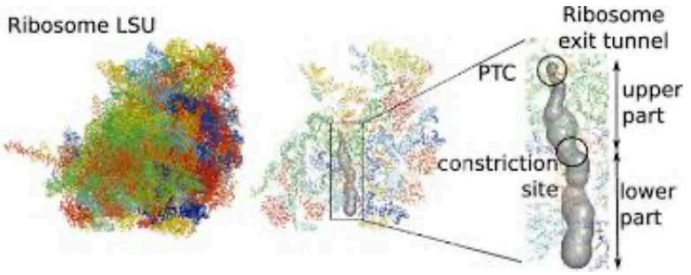



Figure 2: A ribosome, showing the ribosome exit tunnel (Duc, Batra, Bhattacharya, Cate, & Song, 2019)

In co-translational folding, the ribosome provides a highly structured environment that promotes proper folding as the polypeptide chain emerges. Proteins begin to form secondary structures inside the exit tunnel of the ribosome. The tunnel is about 100 Å long and 10–30 Å wide (Samatova et al., 2023). The size of the tunnel restricts the protein's ability to fold prematurely or adopt incorrect structures. This tunnel, which is only large enough to accommodate a few amino acids at a time, ensures that the folding process is gradual and controlled. Furthermore, this confinement helps facilitate the hydrophobic collapse—a step in the protein folding process where hydrophobic (water-repelling) amino acid residues come together in the protein's interior to form a compact core (Rich, 2007). The ribosome's tunnel, by limiting the available space, may guide this process, helping to expose hydrophobic regions at the right moment while preventing their exposure to the aqueous cellular environment.

The tight confines of the ribosomal exit tunnel also serve to coordinate the folding of the nascent polypeptide with the rate of translation. The speed at which the ribosome moves along the mRNA dictates the time scale over which the protein emerges and begins to fold (Samatova et al., 2023). As the polypeptide extends, it interacts with the tunnel walls, which helps control the rate of compaction. This coordination ensures that folding begins in a spatially constrained manner, preventing the protein from adopting non-native structures before sufficient sequence information has been synthesized.

Moreover, the ribosome-associated chaperones (proteins that assist in protein folding) often interact with the nascent protein as it exits the tunnel (Samatova et al., 2023). These chaperones bind to specific regions of the protein, stabilizing it as it folds into its correct configuration. They can help to prevent improper folding or aggregation by stabilizing the partially folded intermediates that form within the exit tunnel. This combination of spatial restriction and chaperone assistance provides a powerful mechanism for ensuring that proteins fold correctly and efficiently while still attached to the ribosome.

Another critical aspect of the exit tunnel is its ability to adapt dynamically to the needs of the protein being synthesized. Recent studies from Samatova et al. have suggested that the shape and size of the exit tunnel may vary depending on the specific protein being synthesized. For example, larger, more complex proteins might encounter different structural features of the tunnel compared to smaller proteins, which could influence how they fold.

This adaptability suggests that the ribosome provides a responsive folding environment. It not only offers a spatially constrained space but also adjusts to accommodate the specific folding requirements of different nascent proteins (Samatova et al., 2023). In some cases, the ribosome itself might actively contribute to the folding process by making specific structural adjustments or by modulating the translation rate in response to the protein's folding needs. For instance, if the emerging protein requires more time to adopt a critical fold, the ribosome may slow its translation rate, allowing the protein to fold more effectively within the confines of the exit tunnel.

## THE THERMODYNAMICS OF CO-TRANSLATIONAL FOLDING

Recent experiments using nuclear magnetic resonance (NMR) spectroscopy and simulations of ribosome-nascent chain complexes (RNCs) have provided new insights into how the ribosome influences protein folding on a molecular level. In multiple studies within the Nature Publishing Group, researchers purified ribosome-nascent chain complexes and investigated the structural dynamics of the emerging polypeptide using high-resolution techniques. By measuring hundreds of interatomic distances and combining this data with simulations of millions of atoms, they were able to construct experimentally derived models of the nascent chain at different stages of synthesis.

One key observation from these studies is that the unfolded nascent polypeptide on the ribosome is significantly more solvated (i.e., surrounded by water molecules) and structurally expanded than an isolated polypeptide in solution. The ribosome provides a confined space that affects the hydration state of the nascent chain: water molecules bound to the protein have lower entropy (a lower degree of disorder) compared to free water molecules. This reduction in entropy makes the solvated nascent polypeptide more ordered, which in turn reduces the entropy of the entire ribosome-nascent chain complex.

This greater order in the solvated nascent chain on the ribosome contrasts with the higher entropy of freely solvated polypeptides, which are typically more disordered in solution (Nature Publishing Group, 2024). The entropic penalty of folding—a process that reduces the degree of disorder in the protein chain—is typically discouraging when folding occurs in free solution.

However, on the ribosome, the entropic cost of folding is significantly reduced. Since the unfolded nascent chain is already more ordered due to solvation effects in the confined ribosomal environment, the transition to folded intermediates is less entropically costly. This makes co-translational folding (coTF) energetically more favorable, pushing the nascent polypeptide toward stable, soluble, and partially structured conformations even before translation is completed.

As the nascent chain emerges from the ribosome, the protein enters a series of partially folded intermediate states, which are stabilized by the ribosomal environment and its associated factors. These intermediates are a hallmark of co-translational folding, a process in which the protein adopts its functional conformation through a series of sequential steps during translation. The ribosome plays an essential role in shaping the folding process by stabilizing these intermediates and guiding the nascent protein along a defined folding pathway.

In contrast to folding in isolation, where intermediates tend to form transiently and are less stable, co-translational folding promotes the formation of stable intermediates that are thermodynamically more favorable. These co-translational intermediates are typically less prone to misfolding and aggregation, as they are formed in the more controlled, confined space provided by the ribosome (Nature Publishing Group, 2024). As the nascent chain lengthens, the intermediates progressively convert into the fully folded, active structure of the protein.

The ability of the ribosome to stabilize these partially folded states is crucial for efficient protein synthesis. The ribosome's exit tunnel, by constraining the nascent chain, ensures that the polypeptide is not able to fold indiscriminately or prematurely into an energetically unfavorable conformation. Instead, folding occurs sequentially and in a more controlled manner, with each elongation step allowing the protein to move along a defined folding pathway toward its final, active form (Nature Publishing Group, 2024).

The unique structural and solvation environment within the ribosomal exit tunnel also influences the thermodynamics of protein folding. The entropic penalty of folding is lower in the ribosome-bound state compared to isolated polypeptides in free solution, which is a critical factor in promoting the formation of co-translational folding intermediates. This results in a more favorable folding process overall, as proteins can fold in a more efficient and controlled manner while they are still being

synthesized (Samatova et al., 2023).

Interestingly, this finding challenges previous assumptions about how folding on the ribosome works. Contrary to the idea that the ribosome might simply provide a "scaffolding" for protein synthesis, recent evidence suggests that the loss of entropy associated with the solvated unfolded polypeptide on the ribosome is a key mechanism by which the ribosome actively stabilizes partially folded intermediates. These intermediates, which form earlier in the synthesis process, eventually lead to the protein adopting its final functional conformation. This thermodynamic shift pushes the protein towards folding at an earlier stage, aiding in the overall efficiency of protein synthesis.

**IMPLICATIONS**

The understanding of ribosome-mediated protein folding has broad implications for both basic and applied biological research. First, it underscores the efficiency of protein synthesis within cells. By guiding proteins through partially folded intermediates as they are synthesized, the ribosome reduces the chances of misfolding and aggregates forming, ensuring proteins achieve their correct structure more efficiently. This is particularly important for cells with high protein production demands, such as neurons and muscle cells, where rapid and accurate protein folding is crucial for cellular function.

In addition, aberrant protein folding and translation processes are increasingly recognized as factors in cancer development. Cancer cells often exhibit elevated levels of protein synthesis and rely heavily on chaperone systems to maintain proteostasis under stressful conditions. Disruptions in ribosome-mediated folding can contribute to oncogenesis by allowing the accumulation of dysfunctional proteins that promote uncontrolled growth. Targeting the ribosome's role in protein folding could therefore offer novel strategies for cancer therapy, either by selectively impairing the folding of oncogenic proteins or by exploiting vulnerabilities in the cancer cell's heightened dependence on proteostasis mechanisms.

Finally, the ribosome's influence on protein folding also opens new avenues for drug development. By targeting the ribosome's exit tunnel or associated chaperones, researchers could develop therapies that optimize protein folding during translation, potentially preventing misfolding and improving the efficiency of drug discovery efforts related to protein-based diseases. This could lead to the creation of small molecules or biologics that specifically assist in the proper folding of disease-related proteins.

**REFERENCES**

1. Daniel H. Rich. "Stereochemical Aspects of Drug Action I: Conformational Restriction, Steric Hindrance and Hydrophobic Collapse." *The Practice of Medicinal Chemistry (Second Edition)*, Academic Press, 9 May 2007.

2. Ekaterina Samatova 1, et al. "How the Ribosome Shapes Cotranslational Protein Folding." *Current Opinion in Structural Biology*, Elsevier Current Trends, 9 Dec. 2023.

3. Duc, Batra, Bhattacharya, Cate, & Song, 2019. "Differences in the Path to Exit the Ribosome across the Three Domains Of Life."

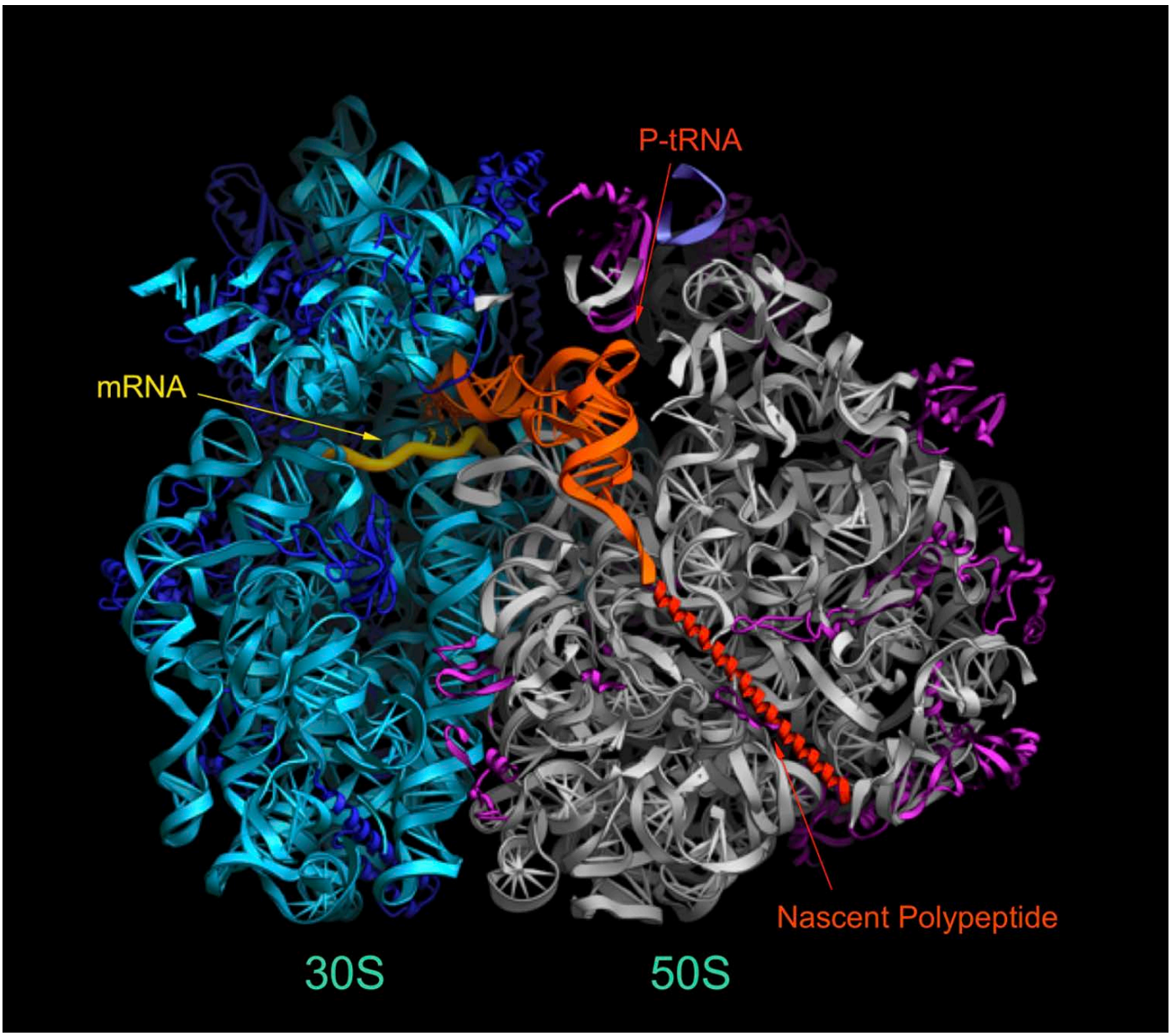
4. "How Ribosomes Shape Protein Folding." *Nature News*, Nature Publishing Group, 9 Oct. 2024.

5. "Hydrophobic Collapse." *Hydrophobic Collapse - an Overview | ScienceDirect Topics*.

6. *Nature News*, Nature Publishing Group.

7. "Ribosome." *British Society for Cell Biology*.

8. "Ribosome." *Genome.Gov*.




A labeled structural model of a bacterial 70S ribosome during translation. The small (30S) subunit is shown in cyan and the large (50S) subunit in gray. mRNA (yellow) threads through the 30S subunit, aligning codons for translation. A tRNA molecule bound to the ribosome's P-site (P-tRNA, purple) is positioned in the peptidyl transferase center of the 50S subunit, with the attached nascent polypeptide (red) extending through the ribosomal exit tunnel. This configuration highlights the coordinated interaction between ribosomal subunits, mRNA, tRNA, and the emerging protein during elongation.

*Image credits: Noller Lab. Center for RNA Science and Therapeutics, University of California, Santa Cruz*

# HOW DOES ANTIBIOTIC RESISTANCE WORK? THE MECHANISMS OF ANTIBIOTIC RESISTANCE

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**ABSTRACT**
Antibiotic resistance occurs when bacteria survive in the presence of antibiotics, which are generally used to kill bacteria. This resistance has become a growing issue in several industries, including healthcare, where bacterial infections have become more difficult to treat. With antibiotic resistance rising to be a threat to the health of humans and animals, it is essential to understand how antibiotics work and how bacteria are able to bypass their effects. Antibiotics primarily serve their purpose by breaking apart the bacteria or interrupting necessary processes for survival. Bacteria, once they acquire resistance through either mutation or uptake of foreign DNA, use many strategies to counteract antibiotics. Currently, there are various approaches being researched to prevent or work around antibiotic resistance. With the increasing prevalence of resistance, this research is crucial to reverse its impact.

## INTRODUCTION

Antibiotics are drugs that are used to kill bacteria and to prevent bacterial growth. In healthcare, antibiotics are commonly utilized to treat bacterial infections. However, improper use of these drugs can lead to a phenomenon known as antibiotic resistance, where some bacteria are able to withstand the effects of antibiotics, furthering growth and proliferation. Such a situation can be deadly to any infected human or animal (U.S. National Library of Medicine, n.d.). Because of its detrimental impact, it is important to understand how antibiotic resistance arises, how it works, and how it can be prevented or bypassed to ensure infections are no longer resistant.

## HOW ANTIBIOTICS KILL BACTERIA

Antibiotics were first discovered as naturally occurring compounds in certain organisms. Penicillin, the first discovered natural antibiotic, was isolated from a mold species (Gaynes, 2017). Today, most antibiotics are chemically modified versions of natural antibiotics (Pancu et al., 2021). Just as bacteria vary in structure, most antibacterial activity is categorized into one of five different mechanisms. Despite being distinct processes, each has a similar effect: killing the bacteria or terminating their growth.

Antibiotics can interrupt bacterial cell wall synthesis. The cell wall in bacterial cells provides structure. An antibiotic can cause this wall to break to prevent cell wall synthesis. As a result, the cell can no longer control its structure. In such a situation, the bacterium is unable to sustain itself (Uddin et al., 2021).

Antibiotics can also inhibit bacterial cell membrane function without breaking down the cell wall. The membrane of a cell is a thin layer within the cell wall primarily composed of lipids with some carbohydrates and proteins attached. The membrane selectively allows certain molecules to enter or exit the cell, which help the cell to perform processes that keep it alive and help it grow. If the cell membrane is not functioning properly, the bacterium cannot control the environment within its cell. The change in environment interferes with the bacterium's own workings, causing the cell to die. Some antibiotics specifically target glycolipids—lipids with an attached carbohydrate—to destroy the membrane (Uddin et al., 2021).

Some antibiotics can disrupt the mechanisms of protein or nucleic acid synthesis, both of which impact the bacteria's well-being in a similar manner. Nucleic acids, such as DNA, are processed to synthesize proteins. Without proper synthesis of nucleic acids, it is impossible to make proteins, which are essential for various functions within the cell. Lacking necessary proteins, the bacteria can no longer stay alive. Additionally, both nucleic acids and proteins are important for the growth and replication of bacteria. If neither of these molecules are synthesized, bacteria cannot proliferate.

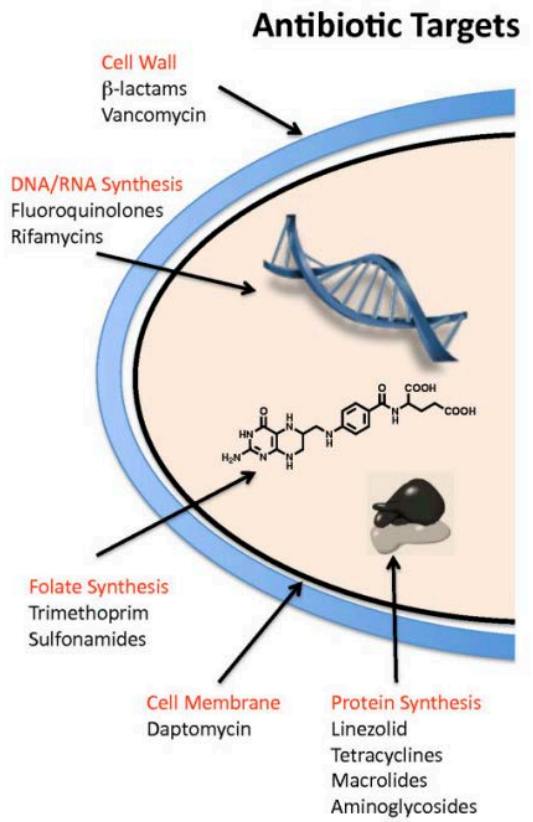



Figure 1: Antibiotic targets in bacteria (Wright, 2010)

Other antibiotics can inhibit metabolic pathways in bacteria through degradation or modification of molecules involved in these processes. Metabolic pathways are ever-present in not only bacterial cells, but in every cell, consisting of all the chemical reactions that keep cells alive, including the conversion of food to energy. By disrupting these processes,

antibiotics effectively block bacteria from sustaining themselves (Uddin et al., 2021). However, in the face of antibiotic resistance, any antibiotic efforts can be deemed useless.

**HOW ANTIBIOTIC RESISTANCE IS ACQUIRED**

There are various mechanisms used by bacteria to protect themselves against antibiotics, but how is this resistance acquired? Bacteria generally use one of two major genetic strategies to attain this resistance against antibiotics: mutations or acquisition of foreign DNA (Munita and Arias, 2016).

Random mutations in DNA can have substantial impacts. Certain bacteria may acquire a mutation that changes how they interact with the antibiotic, which may cause the antibiotic to be less effective. As some bacterial cells attain this mutation, they survive against the antibiotics and proliferate. The cells without the mutation are still killed. Consequently, this gives the resistant bacteria more resources, such as habitat, space, and nutrients, to grow at an even faster rate. Eventually, most of the cells will be mutated to be resistant to the antibiotic (Munita and Arias, 2016).

Another way bacteria can attain this resistance is through horizontal gene transfer. Horizontal gene transfer is the movement of genetic information between two distinct bacteria. This could mean that two bacteria physically connect, allowing DNA to move from one organism to another. A bacterium can also uptake DNA floating in its environment. In nature, this DNA comes from dead bacteria, which release their contents outside of the cell (Kloos et al., 1994). Any of these methods would lead to the exchange of genetic material amongst bacteria that are not related through a parent-offspring connection. This allows bacteria with resistance to pass on their ability to other cells. This way, an increasing amount of cells can acquire the resistance.

**THE DIFFERENT MECHANISMS OF ANTIBIOTIC RESISTANCE**

There are four broad categories of processes through which bacteria act upon their resistance to antibiotics: modifying the antibiotic molecule, preventing the antibiotic from reaching its target, changing target sites, and undergoing global cell adaptations. Each of these categories can be further broken down into specific mechanisms.

Bacteria, once resistant, can modify the antibiotic through chemical change to prevent it from harming the cell. In this process, the bacterial cell acquires a gene that produces certain enzymes, which are proteins with catalytic functions. Enzymes are able to speed up or push certain chemical reactions. In this case, the enzymes can change the antibiotic molecule so that it no longer functions. Some bacteria produce enzymes that completely destroy the antibiotic molecule. As of now, it is thought that there are over 1,000 different enzymes capable of this function (Munita and Arias, 2016).

Bacteria are also able to prevent the antibiotic from penetrating the cell wall or efflux the antibiotic out of the cell. Many antibiotics target areas in the cell membrane, meaning these antibiotics need to penetrate the membrane in order to perform their function. Several bacteria develop efficient membranes that act as barriers against antibiotic uptake. Other bacteria develop complex machines in the bacteria cell membrane that work to extrude toxic compounds out of the cell, known as efflux pumps, which work against antibiotics. These pumps can have strict or broad substrate specificity, which refers to the range of molecules the pump is able to recognize (Munita and Arias, 2016). In these ways, the cell is able to protect the cell membrane from antibiotics, which, in turn, keeps the bacterium alive.

In the case of target-specific antibiotics, which act at a specific target site on the bacterial cell, the bacterium is able to interfere with the site to achieve resistance. One method is to protect the target. Through this, the bacteria contain molecules that bind to the target site, preventing the antibiotic from reaching it. Bacteria can also modify the target site, preventing the antibiotic from recognizing the site. Target site modification can be achieved by inducing mutations in the genes that encode the site. It can also be achieved by using enzymes to chemically alter it, or by replacing the original target with a different one (Munita and Arias, 2016). Any of these methods work to deter the antibiotic from interacting with its

target site, allowing bacteria to resist the effects of the antibiotic.

Lastly, bacteria can go through global cell adaptations that lead to antibiotic resistance. Through generations of evolution, bacteria can develop ways to deal with environmental pressures to survive against detrimental environments. For example, bacterial organisms inside of a host are constantly attacked by the host's immune system. Over time, these bacteria are able to adapt to the stress in their environment and develop complex mechanisms to prevent the disruption of important cell processes, such as cell wall synthesis and membrane homeostasis (Munita and Arias, 2016). After many years of adaptation and evolution, bacteria can survive hostile conditions, including those created by antibiotics.

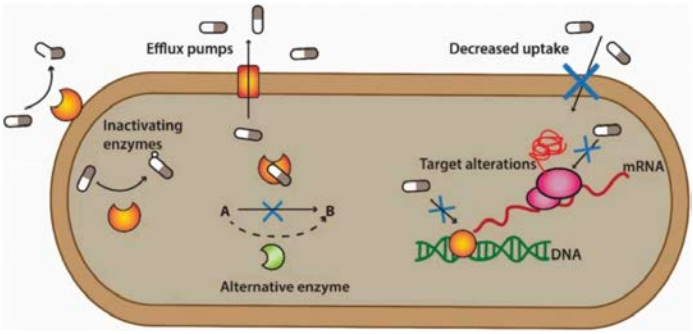



Figure 2: Mechanisms of antibiotic resistance in bacteria (Mutuku et al., 2022)

## STRATEGIES TO COMBAT ANTIBIOTIC RESISTANCE AND THE FUTURE

It is important to understand how antibiotic resistance works in order to combat its harmful effects. Although many of the mechanisms of antibiotic resistance are known, there are many that are yet to be discovered. Despite this, researchers are working to find ways to prevent and reverse antibiotic resistance, which has been emerging quickly.

One researched method to counter antibiotic resistance is to use the CRISPR-Cas9 system. CRISPR-Cas9 can be used to detect and modify genes that confer antibiotic resistance to deactivate. Currently, research has shown that CRISPR-Cas9 can change the structure of bacteria. In a study by Citorik and collaborators, a mouse model was used to determine how well Cas9 can be used against bacteria (Uddin et al., 2021). Cas9 is a protein that functions as molecular scissors to modify genes. Through this study, it was determined that CRISPR-Cas9 can work in certain situations. Unfortunately, as the complexity of bacterial communities increases, it becomes difficult to use these methods accurately and successfully.

An important part of the research to combat antibiotic resistance has been the use of bioinformatics, which includes all the different models that are used to identify and understand molecules. Many methods in the field of bioinformatics can be insightful about bacteria and antibiotic resistance, some of which are currently being used to develop drugs against bacteria with antibiotic resistance. One of these methods is whole genome sequencing (WGS): the use of various methods to analyze the entire genome, or DNA, of the bacteria. This provides information about how the DNA codes for various proteins that lead to resistance (Uddin et al., 2021). WGS allows us to understand the bacterial genome so that drugs can effectively target the correct proteins related to antibiotic resistance. Another method within bioinformatics is using machine learning tools to predict antibiotic resistance. WGS can be used along with prior knowledge to utilize already understood pathways towards resistance to predict new pathways and mechanisms.

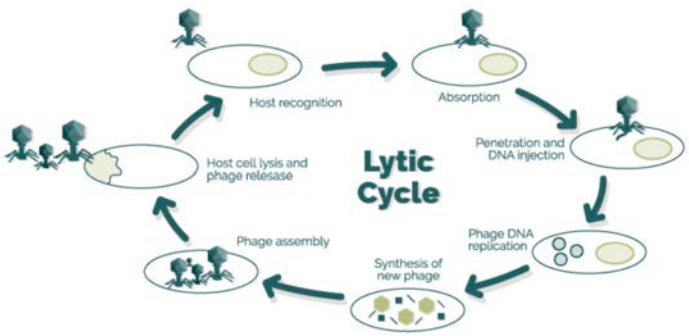



Figure 3: Bacteriophage infection and lysis of bacteria (Coliphages, 2024)

Another found method to combat antibiotic resistance involves the use of bacteriophages, which are viruses that infect bacteria. Phages are used to insert antibiotics directly into target

bacteria without affecting surrounding cells. Many phages are lytic, meaning they enter the bacteria, use the bacteria's resources to replicate, and then exit the bacteria, leaving it to die (Uddin et al., 2021). Moreover, in another study, it was found that the use of bacteriophages along with antibiotics led to more successful control of bacterial growth than either method alone (Łusiak-Szelachowska et al., 2022). These methods, if specified to be unharmful to cells that are not bacteria, may be a powerful alternative or additive to antibiotics.

**CONCLUSION**

As antibiotic resistance becomes more prominent in bacteria, it is critical to find alternative methods. Antibiotics are a common measure to kill bacteria in various fields. Unfortunately, increased usage of antibiotics has led to difficulties in battling antibiotic resistance, which leads to continuing problems in healthcare as diseases and infections are left without effective treatment.

Antibiotic resistance may be a random occurrence, but it is one that adapts quickly through populations of bacteria. Through antibiotic resistance, bacteria are able to survive, replicate, and infect. This prevalent resistance makes it crucial to understand how bacteria acquire this resistance and how it can be prevented or bypassed through other methods.

**REFERENCES**

1. Coliphages. (2024). How do bacteriophages reproduce?.

2. Gaynes, R. (2017). The discovery of penicillin—new insights after more than 75 years of clinical use. Emerging infectious diseases, 23(5).

3. Kloos, D. U., Strätz, M., Güttler, A., Steffan, R. J., & Timmis, K. N. (1994). Inducible cell lysis system for the study of natural transformation and environmental fate of DNA released by cell death. Journal of bacteriology, 176(23), 7352-7361.

4. Łusiak-Szelachowska, M., Międzybrodzki, R., Drulis-Kawa, Z., Cater, ... & Górski, A. (2022). Bacteriophages and antibiotic interactions in clinical practice: what we have learned so far. Journal of Biomedical Science, 29(1), 23.

5. Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Virulence mechanisms of bacterial pathogens, 481-511.

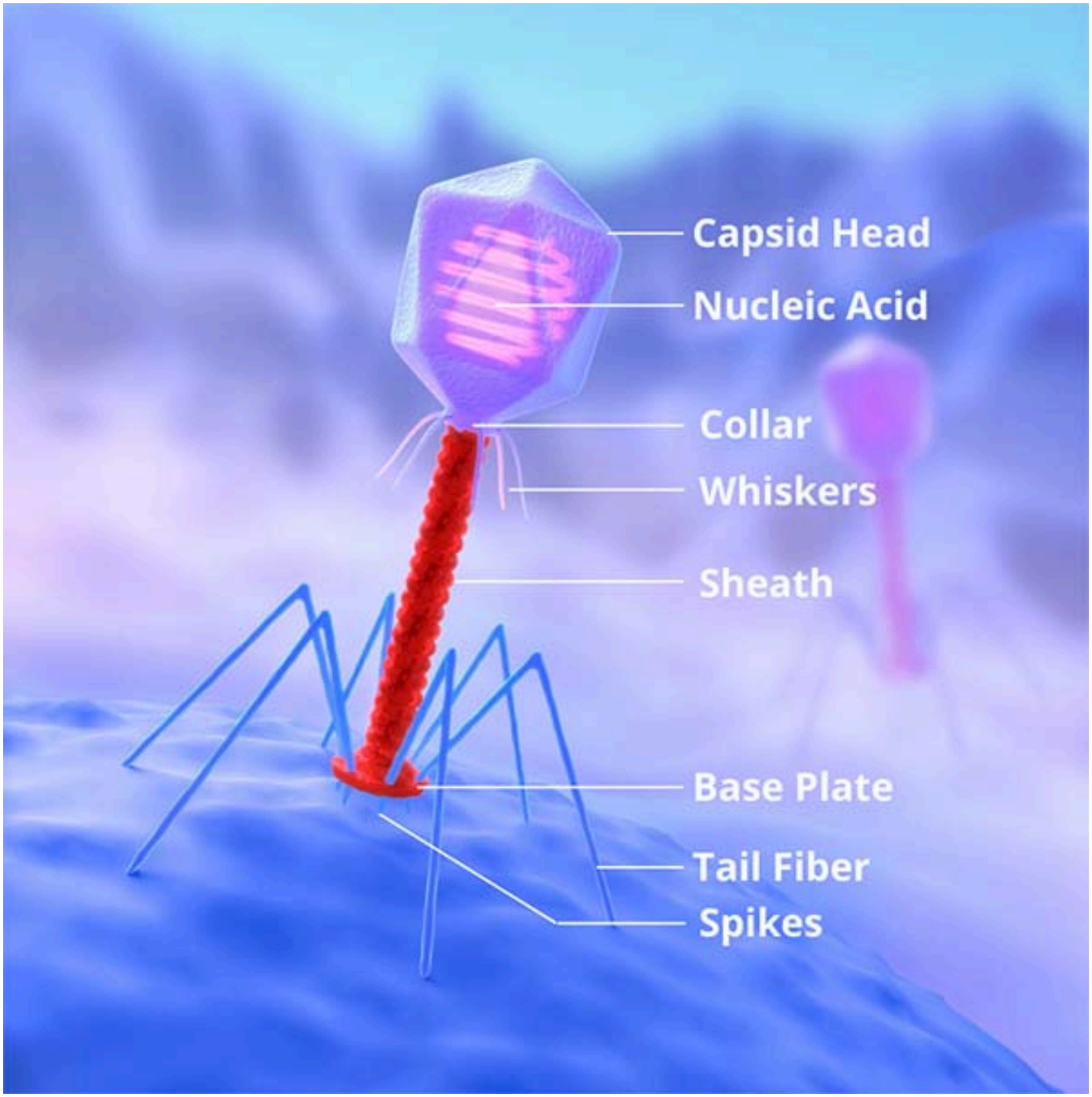
6. Mutuku, C., Gazdag, Z. & Melegh, S. Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. World J Microbiol Biotechnol 38, 152 (2022).

7. Pancu, D. F., Scurtu, A., Macasoi, I. G., Marti, D., Mioc, M., Soica, C., ... & Dehelean, C. (2021). Antibiotics: conventional therapy and natural compounds with antibacterial activity—a pharmaco-toxicological screening. Antibiotics, 10(4), 401.

8. Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., ... & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of infection and public health, 14(12), 1750-1766.

9. U.S. National Library of Medicine. (n.d.). Antibiotic resistance. MedlinePlus.

10. Wright, G.D. Q&A: Antibiotic resistance: where does it come from and what can we do about it?. BMC Biol 8, 123 (2010).




The structure of a T4 bacteriophage. With its characteristic icosahedral head, helical tail, and spider-like tail fibers, the T4 phage is a classic example of lytic bacteriophages being explored for phage therapy in combination with antibiotics to combat antibiotic-resistant bacterial infections.

*Image citation: "The Most Spooktacular Virus of All: The Bacteriophage." Norgen Biotek Corp., 27 Oct. 2022*

# ANTIMICROBIAL RESISTANCE'S IMPACT ON THE HEALTHCARE SYSTEM: THE ERA OF ANTIBIOTIC ABUSE

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**ABSTRACT**
Nearly a century after the first discovery of antibiotics, the rampant misuse of antibiotics has increased the rate of antimicrobial resistance (AMR) exponentially. This misuse has impacted modern healthcare systems around the world with the rise of drug-resistant infections. While bacterial species have had the ability to resist antibiotics before humans utilized antibiotics for treatment, AMR has increased with overuse of antibiotics to treat bacterial infections (Larsson, 2021). This paper explores the rise of AMR as the consequence of the overuse of antibiotics and the halt in production of new pharmaceuticals. These obstacles drive the increase of AMR, and consequently, the rise of deadly antibiotic-resistant infections such as *Staphylococcus aureus* in modern healthcare systems. In addressing this rise of deadly infection, the international health community has mobilized in fighting AMR through investment in research and development of new antibiotics. The world's reliance on antibiotics is emerging as one of the largest global health threats of the twenty-first century, with impacts felt around the globe.

**WHAT IS ANTIBIOTIC RESISTANCE (AMR)?**

First discovered by Sir Alexander Fleming, antimicrobials were recognized to pose great risks to the public after Fleming warned of the potential for misuse and resistance as early as 1945, "...public will demand [the drug and] ... then will begin an era... of abuses." (Ventola, 2019). Antibiotic resistance is a subset of Antimicrobial resistance (AMR) which occurs when microbes such as viruses, bacteria, fungi, or parasites are no longer affected by the use of antimicrobials, or medicines utilized to prevent and treat infectious diseases in humans, animals, and plants (World Health Organization, 2023).

Figure 1: Synthetic production of penicillin by Professor Alexander Fleming, who first discovered the mould *penicillin notatum,* in his laboratory at St Mary's, Paddington, London (1943) (Imperial War Museums, 2023)

AMR is a natural occurring process in genetic evolution, as bacteria and other microbes evolve over time to resist treatment and compete with other bacteria for resources. In fact, every natural, synthetic, and semi-synthetic antibiotic has been met with resistance from the pathogens they target, signifying the natural need in microbials for resistance (Larsson, 2021). However, AMR rates are being driven forward through the overuse of antibiotics in treating infections and the underproduction of new antibiotics. Data on antibiotic prescriptions reflect the healthcare's heavy reliance on those drugs. In the U.S., for example, the IMS Health Midas database estimated that 22 standard units (e.g., pills, capsules, or doses) of antibiotics were prescribed per person in the 2010 (Ventola, 2019). This study discloses the dependency that healthcare systems have for antibiotic treatments, which has led to an increased rate in AMR through evolution of microbes. Furthermore, of the sheer amount of antibiotics prescribed, studies have shown that many of these antibiotics were incorrectly prescribed. In fact, it has been estimated that thirty to sixty percent of antibiotics prescribed in intensive care units have been found to be unnecessary, inappropriate, or suboptimal to patient recovery. In such cases, incorrect prescription of antibiotics exposes patients to potential complications of antibiotic therapy, and an increased hospital stay (Ventola, 2019). This overuse and misuse of antibiotics have significantly increased AMR rates throughout the world by driving forward genetic evolution of microbes.

While misuse and overuse of antibiotics are significant contributing factors to AMR in hospital or clinical settings, drug production and research in pharmaceutical settings is another contributing factor. A standstill in production of new antibiotics in the pharmaceutical industry has risen in the past decades, with fifteen of the eighteen major pharmaceutical companies abandoning the production of new antibiotics. This arose out of fear of profitability of antibiotics, as antibiotics are for short-term infections, compared to chronic conditions such as diabetes, asthma, or psychiatric disorders (Golkar, 2013). Furthermore, antibiotics are generally less expensive, with new antibiotic courses costing an average of one thousand to three thousand dollars, compared to drugs such as chemotherapy drugs that cost upwards of tens of thousands of dollars (Ventola, 2019). Challenges in production also come from stringent guidelines set by the US Food and Drug Administration in trials for new antibiotic production (Golkar, 2013). After the FDA (the US Food and Drug Administration) approved the drug Telithromycin, it was found to cause hepatotoxicity. Public outcry erupted, prompting the FDA to tighten drug trial purity standards, including antibiotic trials. This resulted in changes in the design of antibiotic trials. For instance, trials were forced to increase their sample sizes to rule out the placebo effect in results. Although later FDA

analysis suggested that smaller sample sizes were sufficient, the requirement is kept, causing unnecessary increase in trial costs and burden on research companies (Shlaes, 2013). Combined with the disadvantages set forth by profitability of chronic illness drugs, many pharmaceutical companies have abandoned production of new antibiotics, increasing resistance of drugs already in circulation.

## IMPACT OF AMR IN COMMON INFECTIONS

Antibiotics are integral in treating common infections throughout the world and were revolutionary for modern medicine. But what happens when common infections start resisting antibiotics? One major effect is death: in a study done by the CDC, it is approximated that seventy thousand deaths from drug-resistant infections occur in the US per year (Golkar, 2013). In 2021, this number increased to one-hundred and thirty thousand deaths in the US, even with the disease- control measures set by the Covid-19 pandemic (Institute, 2024). Globally, this number was almost five billion deaths in 2019 (World Health Organization, 2023). Furthermore, scientists estimate that during the next twenty-five-year span, an average of 39 billion people are expected to perish from drug-resistant infections globally (Barron,2024). This growing crisis highlights the severe consequences of AMR, leaving patients susceptible to common infections. Because AMR compromises the immune systems of patients fighting common infections, this may force physicians to use last-resort medicine, which could have potentially fatal side effects (Dadgostar, 2019). Methicillin resistance in *Staphylococcus aureus* (MRSA), one of the most well-known AMR cases, has recorded high mortality for several years. When comparing the number of deaths every year by MRSA, a study by the CDC revealed that more Americans are killed every year by MRSA than emphysema, HIV/AIDS, Parkinson's disease, and homicide combined (Golkar, 2013). Globally, AMR infections such as MRSA have been found in every single nation. For example, in 2022, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) reported resistance rates in seventy-six countries for forty-two percent of third generation Cephalosporin-resistant *E. Coli* and thirty-five percent for Methicillin- resistant *Staphylococcus aureus* (World Health Organization, 2023). These studies illustrate the significant effects AMR has on once-common infections and infections still rampant in the international health community.

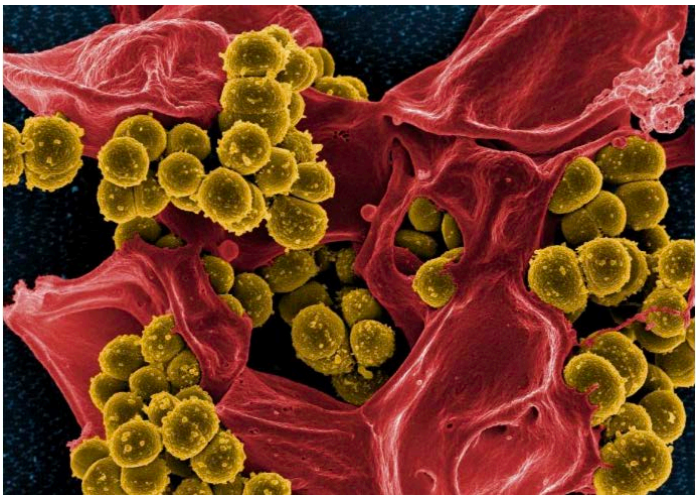


Figure 2: Colorized scanning electron micrograph of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria (gold) interacting with a human neutrophil (red). Image captured at NIAID's Rocky Mountain Laboratories (RML) in Hamilton, Montana (NIAID, 2023)

## WHAT IS AMR COSTING HEALTHCARE?

AMR has significant costs for both health systems and national economies overall. It creates a need for more expensive and intensive care, affects productivity of patients or their caregivers through prolonged hospital stays, and affects life-saving procedures. When AMR infections are identified, health care providers may be forced to prescribe last-resort drugs, which are usually more expensive, and as these last-resort drugs become less effective, it becomes more likely the infection will become untreatable. For example, *Klebsiella pneumoniae,* a common intestinal bacterium, has shown enough resistance for the last-resort class of drug called carbapenem to be utilized (World Health Organization, 2023). As more drugs become ineffective in treating AMR infections, increasingly expensive and risky treatments will be implemented.

Consequently, fighting AMR infections has significantly cost healthcare systems and global economies billions of dollars annually. In fact, fighting AMR infections costs the US healthcare system an estimated twenty-one to thirty-four billion dollars per year (Golkar, 2013). Hospital and clinical settings may have to take extra

steps when treating AMR infections which, on average, add an additional fourteen hundred dollars to hospital bills for patients fighting bacterial infections. Excessive costs come from expensive and intensive treatments with prolonged hospital stays and the use of intensive care units (ICUs) to prevent further spread of infection (Dadgostar, 2019). In many cases, physicians must use valuable time and energy to experiment with available drugs for treatment which increases hospital stays and if there is an outbreak in a hospital of AMR infection, an entire wing or hospital could be forced to quarantine.

Along with the direct effects of fighting AMR infection, AMR also affects the ability of healthcare workers to do procedures that involve antibiotics, as the risk of infection is increased in operations such as organ transplantation, hip replacement, C-sections, and chemotherapy (World Health Organization, 2023). In organ transplantation, both the receiver and donator of said organ are exposed to possible infections during surgery, and drug-resistant pathogens increase the risk of death and transplant failure of the organ. Furthermore, chemotherapy weakens the patient's immune system, which exposes the patient to AMR infections. Therefore, physicians will not give antibiotics to chemotherapy patients if AMR is prevalent (Dadgostar, 2019). Many of the treatments affected by AMR infections are vital to the healthcare system, and without protection of these procedures, healthcare systems around the world will and are struggling to provide quality healthcare to its citizens.

## GLOBAL INITIATIVES TO CURB AMR

With the significant effects of AMR already taking hold in modern healthcare systems, multiple global organizations have formed to combat it since the early 2000s. One of these global organizations is the Quadripartite Joint Secretariat on Antimicrobial Resistance, which is a partnership between the following organizations: the World Health Organization, Food and Agriculture Organization of the United Nations, the United Nations Environment Program (UNEP) and the World Organization for Animal Health (WOAH). This cohort focuses on fighting the spread of AMR through the design, communication, implementation, and monitoring of legislation and policies concerning AMR. These focuses are part of the Global Action Plan (GAP) on Antimicrobial Resistance which was adopted by the World Health Assembly in 2015 (World Health Organization, 2023). With this plan, the global health community is united in its goal of attaining positive health and economic outcomes concerning AMR.

## CONCLUSION

With the overuse and misuse of antibiotics in modern healthcare systems, antimicrobial resistance is on the rise around the globe. Due to the misuse of antibiotics and the standstill of production of new antibiotics driving the rise of AMR, common infections such as *Staphylococcus aureus* are becoming harder to treat, mortality rates for infections are increasing, and healthcare expenses are climbing annually with the increase of AMR infections. However, there are multiple global initiatives forming to create positive health and economic outcomes despite the significant effects of AMR rates increasing. Investment in research in new antibiotic production and aiding in efforts to circumvent reliance on antibiotics is imperative if AMR is to decrease. In order to circumvent deadly outcomes for healthcare worldwide, the global health community must revisit their reliance on antibiotics and the unknowns of how to combat this increasingly perilous issue.

## REFERENCES

1. Barron, M. (2024, October). *The antimicrobial resistance pandemic: Breaking the silence*. ASM.org.

2. Dadgostar, P. (2019, December 20). *Antimicrobial resistance: Implications and costs*. Infection and drug resistance.

3. Golkar, Z., Bagasra, O., & Pace, D. G. (2013, September 10). *Bacteriophage therapy: a potential solution for the antibiotic resistance crisis.*https://jidc.org/index.php/journal/article/view/24518621/994

4. Imperial War Museums. (2024). *Professor Alexander Fleming*. World History Encyclopedia. photograph. https://www.worldhistory.org/image/19117/professor-alexander-fleming/.

5. Institute for Health Metrics and Evaluation. (2024, September). *The Lancet: More than 39 million deaths from antibiotic-resistant infections estimated between now and 2050, suggests first global analysis*. Institute for Health Metrics and Evaluation.

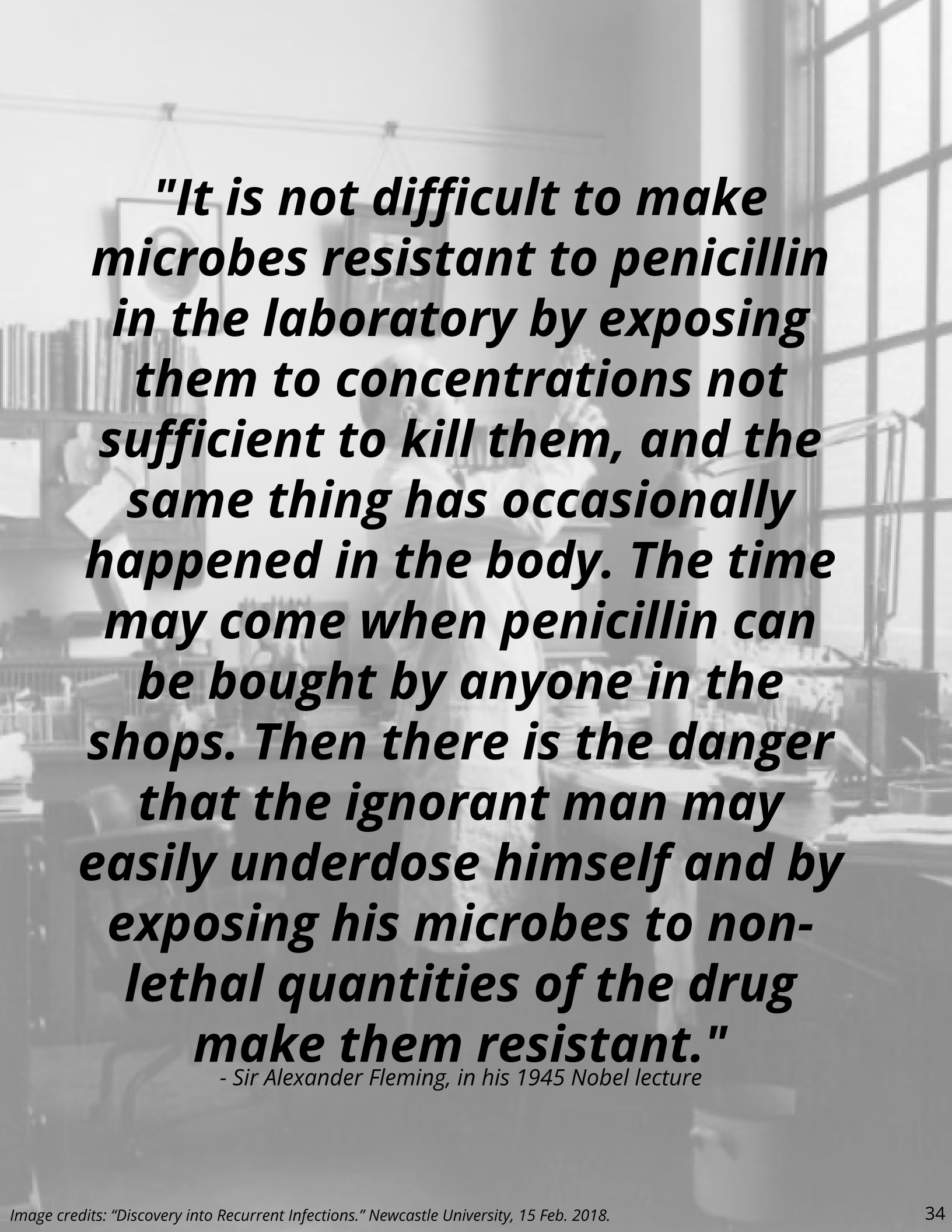
6. Larsson, D. G. J., & Flach, C.-F. (2021, November 4). *Antibiotic resistance in the environment*. Nature News.

7. NIAID. (2023). *Methicillin-Resistant Staphylococcus aureus (MRSA) Bacteria*. flickr. photograph.

8. Shlaes, D. M., Sahm, D., Opiela, C., & Spellberg, B. (2013, October). *The FDA reboot of antibiotic development*. Antimicrobial agents and chemotherapy.

9. Ventola, C. L. (2019, December 20). *The antibiotic resistance crisis: Part 1: Causes and threats*. P & T:
a peer-reviewed journal for formulary management.

10. World Health Organization. (2023, November 21). *Antimicrobial resistance*. World Health Organization.



***"It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant."***

*- Sir Alexander Fleming, in his 1945 Nobel lecture*

*Image credits: "Discovery into Recurrent Infections." Newcastle University, 15 Feb. 2018.*

# GLP-1 RECEPTOR AGONISTS: A POTENTIAL TREATMENT FOR AUD

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**ABSTRACT**
Alcohol use disorder (AUD) is a chronic substance use disorder characterized by uncontrolled alcohol consumption. According to the National Institute on Alcohol Abuse and Alcoholism, it affects roughly 28.9 million people in the US (2024) and was linked to 2.6 million preventable deaths worldwide in 2019 (World Health Organization, 2024). One major roadblock in treating AUD is the limited availability of prescribable medications—only three are currently approved in the United States. Consequently, a major focus of current research is finding suitable therapeutic agents. Recent clinical studies suggest GLP-1 receptor agonists (GLP-1 RAs), which are normally used to treat Type 2 diabetes, may be potential candidates.

## WHAT IS GLP-1?

Glucagon-like peptide-1 (GLP-1) is a peptide hormone vital to regulating energy homeostasis in humans. Derived from proglucagon, GLP-1 is secreted in response to food consumption. It binds to a G-protein coupled receptor called GLP-1R. This triggers a signaling pathway which leads to an increase in an intracellular second messenger molecule known as cAMP, resulting in a variety of downstream effects including regulation of insulin secretion, glucagon secretion, and proliferation of pancreatic β cells (Zheng et al., 2024). Essentially, these changes lead to decreased levels of glucose in the blood, feelings of "fullness," and slowed digestion.

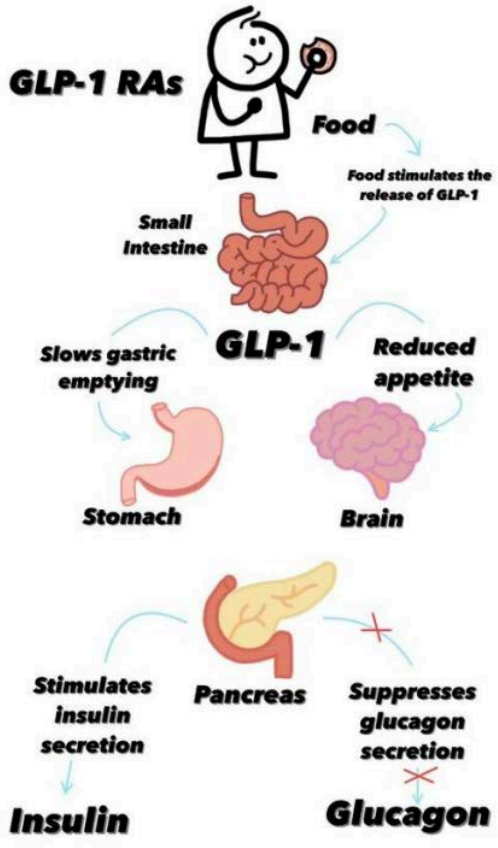



Figure 1: A diagram summarizing the process through which GLP-1 modulates hunger and digestion, which involves several different pathways in different areas of the body (Myluckynumber7, 2024)

## GLP-1 RECEPTOR AGONISTS REDUCE ALCOHOL CRAVINGS

A recent randomized clinical trial showed that patients with AUD who were treated with low doses of semaglutide, a GLP-1 receptor agonist, experienced significantly reduced weekly alcohol cravings (Hendershot et al., 2025). GLP-1 receptor agonists are structurally modified versions of naturally produced GLP-1. Changes to specific amino acids in human GLP-1 endow these synthetic peptides with a variety of advantages, including less susceptibility to degradation by dipeptidyl-peptidase-4, which typically breaks down GLP-1, leading to longer-lasting effects (Zheng et al., 2024). These drugs have conventionally been used to treat type 2 diabetes due to their stronger ability to modulate hunger and blood-glucose levels compared to naturally produced GLP-1. This clinical study underscores other preclinical and observational studies which suggest GLP-1 plays an important role in regulating dopamine homeostasis in response to nutrient consumption. Multiple studies have shown that both systemically and locally administered GLP-1RAs can reduce the dopamine release triggered by alcohol in the nucleus accumbens region of the brain, which is part of the reward system neural circuitry (Vallöf et al., 2015; Egecioglu et al., 2013).

## MECHANISTIC THEORIES AND A PROMISING OUTLOOK

However, the precise mechanism of how GLP-1RAs modulate the reward system is still under investigation. One theory is that GLP-1RA stimulation may lead to increased expression of dopamine transporter (DAT) in the brain, which has been tested in rodents (Reddy, 2016). Dopamine transporter is a protein which essentially promotes the reuptake of dopamine back into the neuron from which it was secreted. So far, there has been conflicting evidence to support this theory, with a few studies finding no such effect (Fortin, 2017). Other theories suggest presynaptic and postsynaptic mechanisms are both involved in the role of GLP-1 in the reward system (Kruse Klausen et al., 2022). Despite the uncertainty of how GLP-1 may modulate the reward system, there is increasing interest in adapting current GLP-1RAs to help treat substance use disorders, and several more clinical studies are underway which hope to assess patient outcomes. Much of the GLP-1RA discussion to this date has centered around their ability to treat type 2 diabetes, but these emerging discoveries could potentially lead to the development of a powerful tool to treat AUD.

**REFERENCES**


1. Egecioglu, E., Steensland, P., Fredriksson, I., Feltmann, K., Engel, J. A., & Jerlhag, E. (2013). The glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviors in rodents. Psychoneuroendocrinology, 38(8), 1259–1270.

2. Fortin, S. M., & Roitman, M. F. (2017). Central GLP-1 receptor activation modulates cocaine-evoked phasic dopamine signaling in the nucleus accumbens core.
Physiology & behavior, 176, 17-25.

3. Hendershot, C. S., Bremmer, M. P., Paladino, M. B., Kostantinis, G., Gilmore, T. A., Sullivan, N. R., Tow, A. C., Dermody, S. S., Prince, M. A., Jordan, R., McKee, S. A., Fletcher, P.J., Claus, E. D., & Klein, K. R. (2025). Once-Weekly Semaglutide in Adults With Alcohol Use Disorder: A Randomized Clinical Trial.
JAMA psychiatry, e244789. Advance online publication.

4. Kruse Klausen, M., Thomsen, M., Wortwein, G., & Fink‐Jensen, A. (2022). The role of glucagon‐like peptide 1 (GLP‐1) in addictive disorders.
British Journal of Pharmacology, 179(4), 625–641.

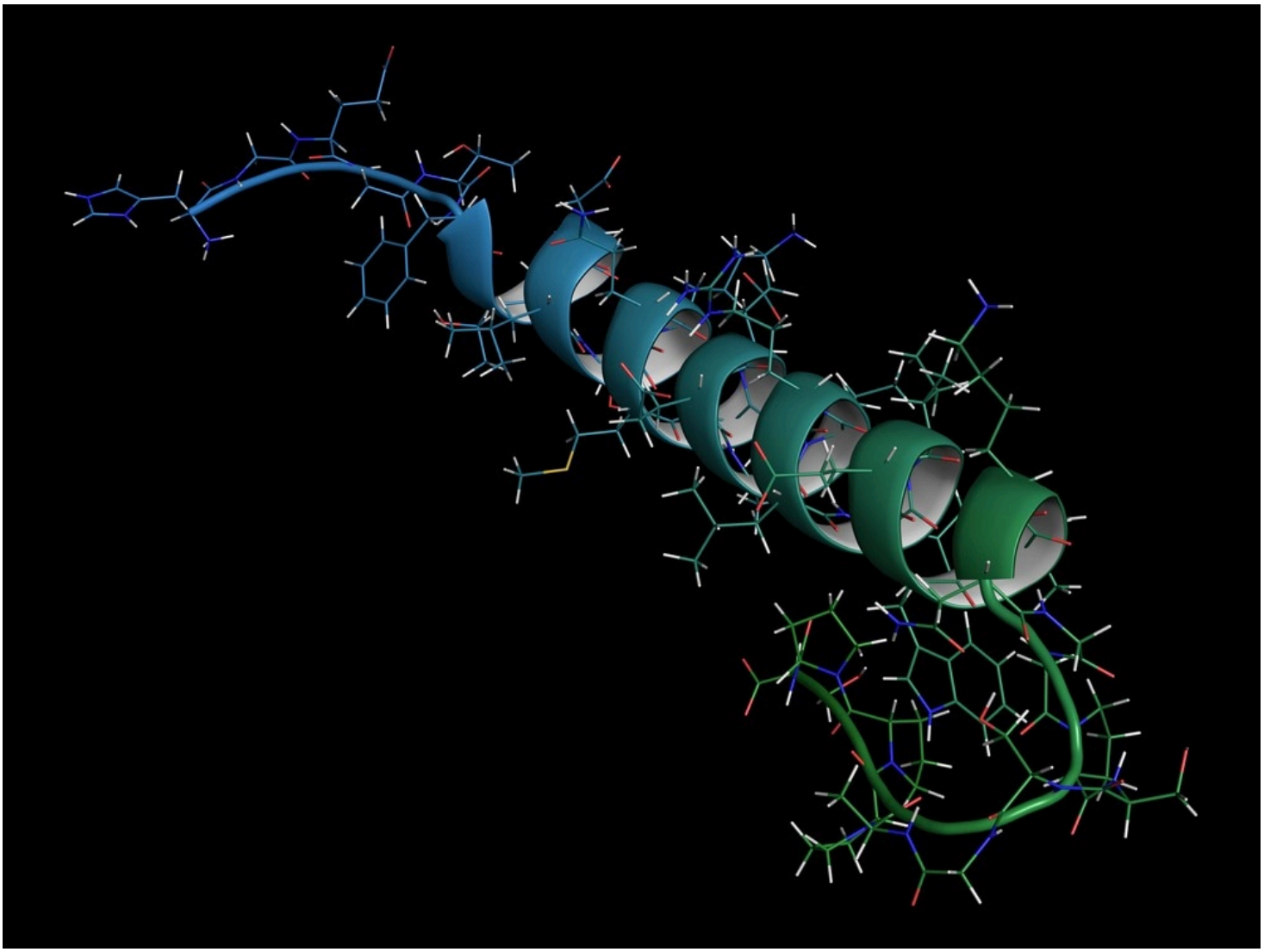
5. Myluckynumber7. (2024, June 22). GLP-1 Signaling in the Body [Illustration].Wikimedia Commons. National Institute on Alcohol Abuse and Alcoholism. (2024, September). Alcohol use disorder (AUD) in the United States: Age groups and demographic characteristics. National Institute on Alcohol Abuse and Alcoholism.

6. Reddy, I. A., Pino, J. A., Weikop, P., Osses, N., Sørensen, G., Bering, T., ... & Galli, A. (2016). Glucagon-like peptide 1 receptor activation regulates cocaine actions and dopamine homeostasis in the lateral septum by decreasing arachidonic acid levels. Translational psychiatry, 6(5), e809-e809.

7. Vallöf, D., Maccioni, P., Colombo, G., Mandrapa, M., Jörnulf, J. W., Egecioglu, E., Engel, J. A., & Jerlhag, E. (2015). The glucagon‐like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. Addiction Biology, 21(2), 422–437.

8. World Health Organization. (2024, June 25). Over 3 million annual deaths due to alcohol and drug use, majority among men. Who.int; World Health Organization: WHO.

9. Zheng, Z., Zong, Y., Ma, Y., Tian, Y., Pang, Y., Zhang, C., & Gao, J. (2024). Glucagon-like peptide-1 receptor: Mechanisms and advances in therapy. Signal Transduction and Targeted Therapy, 9(1), 1–29.



A structural model of a GLP-1 receptor agonist bound in its active α-helical form. Recent findings published in *Nature Medicine* mapped the effects of GLP-1RA use across 175 health outcomes, highlighting significant reductions in risks for cardiovascular events, respiratory failure, neurocognitive disorders, and substance use, alongside increased risks for gastrointestinal and renal side effects. The study supports the broad therapeutic potential, and complexity, of GLP-1RAs in managing chronic disease.

*Image credits: "Researchers Create an Atlas of Health Associations for GLP-1 Receptor Agonists." News-Medical.net, 20 Jan. 2025*
*Journal Reference: Xie, Y. et al. (2025) Mapping the effectiveness and risks of GLP-1 receptor agonists. Nature Medicine.*

# PHYSIOLOGICAL CHANGES AS A RESULT OF THE LSVT BIG AND LOUD PROGRAMS FOR THOSE AFFECTED BY PARKINSON'S DISEASE

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**ABSTRACT**
Parkinson's disease (PD) is a progressive neurological disorder primarily affecting motor function through the degeneration of dopaminergic neurons in the substantia nigra. Additional brain changes include Lewy body accumulation, impaired thalamic and cortical activity related to tremor and rigidity, and degeneration of the limbic and prefrontal regions contributing to mood disorders. Environmental factors like pesticide and metal exposure, alongside genetic variants have been linked to increased PD risk. Neuroplastic changes induced by the Lee Silverman Voice Treatment (LSVT) programs, particularly LSVT LOUD, increase activity in the right motor cortex, auditory cortex, and dorsolateral prefrontal cortex, improving speech production. LSVT BIG promotes cortical and sensorimotor pathway adaptations through large movements leading to enhanced balance and gait. Still, the LSVT program lacks accessibility, long-term effectiveness and patient-friendliness and thus, continued efforts must be made to feasibly implement this therapy.

## INTRODUCTION

Parkinson's disease (PD) is a complex, progressive neurologic disorder often characterized by symptoms including bradykinesia, asymmetric tremor, imbalance, stiffness and potentially mood disorders (American Association of Neurological Surgeons, 2024). PD is measured in five stages, which diagnose a patient's progression based on mobility and the degree to which PD interferes with their day-to-day tasks.

In the United States, about one million people live with Parkinson's disease and 90,000 cases are diagnosed per year. PD is most common in older adults, with about 96 percent of cases diagnosed after the age of 50 (Parkinson's Foundation, 2024). Although more prevalent in men, the primary risk factor for PD is still age. While a majority of PD cases are idiopathic, incidence has also been linked to environmental factors such as pesticide exposure, manganese metal and inorganic pollutants such as polychlorinated biphenyls (PCBs), which are known carcinogens. Thus, PD incidence may be higher in the Rust Belt regions of the United States due to certain industrial activities (Johns Hopkins Medicine, 2019).

A common non-medication treatment program is the Lee Silverman Voice Treatment (LSVT) Program, which includes the LSVT LOUD for vocal function and the LSVT BIG for balance and mobility. The five concepts of the program include:

i. Exclusive focus on voice (specifically vocal loudness)
ii. Stimulation of high-effort productions with multiple repetitions
iii. Intensive delivery of treatment
iv. Enhancing sensory awareness of increased vocal loudness and effort (calibration)
v. Quantification of behaviors (Fox et al, 2002)

It is also important to note that the LSVT programs are used for a variety of other neurologic disorders that fall under the category of parkinsonisms due to its effectiveness (Cleveland Clinic, 2022). While proven to be relatively successful, there are still criticisms of the LSVT programs, which include both methodological and treatment concerns.

## UNDERSTANDING THE NEUROPHYSIOLOGY OF PARKINSON'S DISEASE

Parkinson's disease is rather complex, with neurophysiology varying across patients. While some cases present mild atrophy of the frontal lobe, a vast majority present loss of the substantia nigra pars compacta (SNpc) and locus coeruleus regions near the brainstem. The substantia nigra is primarily responsible for dopamine production, voluntary movement, and cognitive executive functions such as problem-solving. The locus coeruleus (LC) region is involved in attention, vigilance, and stress regulation, and its cAMP pathways are sensitive to chronic stress (Kouli, 2018).

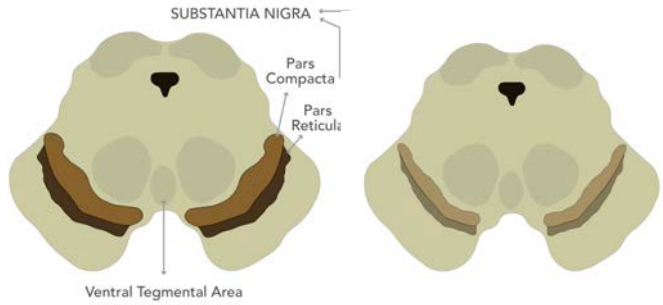


Figure 1: Two depictions of a brain cross section, the one on the left is a typical, healthy patient. while the one on the right is someone with PD. The substantia nigra in the patient with PD is noticeably thinner due to cell death and degeneration of neurons (MNC, 2019)

In many PD cases, the degeneration of dopaminergic neurons in the substantia nigra (Fig. 1), along with cell death in these regions is common. Motor symptoms typically worsen as neuron degeneration increases. This cell loss is responsible for the fundamental motor symptoms of PD and the degeneration of the nigrostriatal pathway, which is a circuit that connects the SNpc to the dorsal striatum and controls motor function (Sonne & Beato, 2022). Furthermore, the accumulation of Lewy bodies, which are abnormal protein clumps, in the SNpc can prevent the production and transmission of dopamine and may cause PD-related dementia.

Specific physical symptoms also present unique brain activity. For patients with an asymmetric

tremor, EEG data in the thalamus, subthalamic nucleus, internal globus pallidus have shown cells firing at tremor frequency. Additionally, studies of cell activity reveal that different cell types are involved in tremors in different regions of the body, which explains why there is no consistent tremor across the body. While both motor and non-motor factors play into gait instability, it appears that abnormal cerebellar function and cognitive multitasking worsen cortical compensation. When looking at rigidity, it seems that the primary factor is excitability changes at the cortical and subcortical levels. More research is also unraveling the relationship between stiffness and long-latency reflexes (Chen et al., 2022).

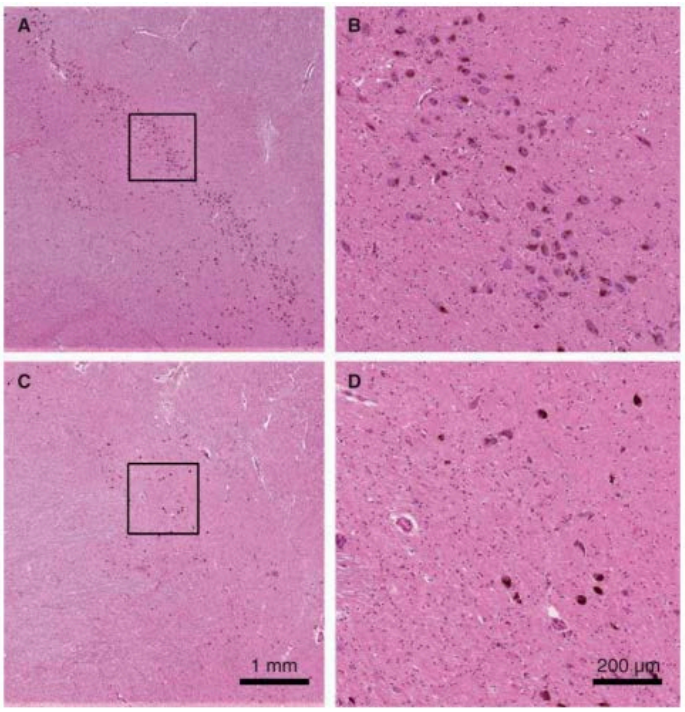


Figure 2: Imaging a coronal section of the substantia nigra pars compacta (SNpc) of a control (A-B) and PD (C-D) brain. Dark brown spots mark dopaminergic neurons. Dopaminergic cell loss is present in the PD brain (Stoker & Greenland)

Mood disorders are also affected by the neurophysiology of PD. In particular, 30-35% of PD patients report depression. This may be related to dysfunction in several regions including the prefrontal cortex, striatal-thalamic-prefrontal cortex circuits, and brainstem indoleamine systems (i.e. dopamine, serotonin and norepinephrine). For those with genetic PD, a few genes have been identified in increasing risk for PD-related depression such as variants in the SLC6A15, TPH2,84 and BDNF genes. In particular, the BDNF gene, or brain-derived neurotrophic factor gene, is primarily responsible for dopaminergic activity and its mRNA expression is significantly reduced in the substantia nigra in PD patients (Howells et al., 2000).

Anxiety disorders have been linked to the loss of function in the fear circuit and limbic cortico-striato-thalamocortical circuit. Notably, the amygdala is a part of the fear circuit and its degeneration is linked to low dopamine output. Some PD patients also experience hallucinations but the mechanism behind this is not understood (Weintraub et al., 2022).

Cell death also occurs in the LC, hypothalamus, olfactory bulb and several other regions that lead to the non-motor symptoms of PD. Because these regions are largely affected by non-dopaminergic neural networks, these symptoms are difficult to treat with typical dopamine replacement therapies (Kouli, 2018).

## DIFFERENCES IN BRAIN PHYSIOLOGY WITH THE LSVT PROGRAMS

### LOUD:

The LSVT LOUD program includes intensive, consistent treatment aimed to improve speech production and recalibrate the brain to recognize vocal volume more accurately (Fox et al., 2006). Exercises include speaking at varying volumes and pitches, reading aloud, and repetition of daily speaking tasks such as answering the phone, ordering food and more. According to a study hypothesizing right-shift theory post-LSVT, there were significant differences in several regions of the brain controlling speech production and understanding.

By treating PD patients with the LSVT program, researchers concluded that their hypothesis was mostly correct, as almost every region in the right hemisphere saw more activity with the program. In particular, the LSVT LOUD program directly modified the right side of the cortical motor, auditory and prefrontal areas as well as the M1-mouth region. The right auditory region is responsible for pitch discernment and timbre discrimination, which the LSVT LOUD program targets through its speech repetition.

Activating the right dorsolateral prefrontal cortex caused a type of "top-down" effect in which other connection regions such as the motor and subcortical connections (basal ganglia - thalamic inputs) improved during speech production. Additionally, the change to thalamic nuclei and the basal ganglia at large can also be attributed to modulation effects from the dorsolateral prefrontal cortex (Narayana et al., 2018).

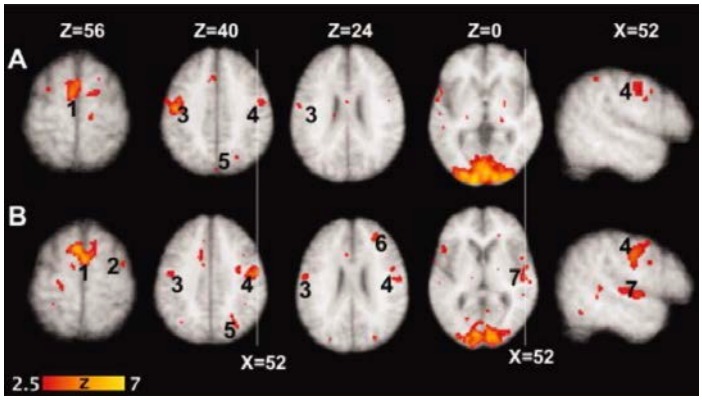


Figure 3: The top row shows brain activity before the LSVT program and the bottom shows brain activity after the program. There is significant increase in activity in the right motor cortex and right superior temporal gyrus, which contains the Wernicke"s area and the primary auditory cortex (Narayana, S. et al., 2018)

In another study, the LSVT program was utilized in children with cerebral palsy (CP). While not under the branch of parkinsonisms, this study provides valuable insights about the potential of the LSVT program to improve neurological conditions. The study discovered that the CP group treated with the LSVT LOUD program displayed increased brain activity (PSC) in the left anterior cingulate gyrus (which controls emotions, motivation and attention) as well as in the supramarginal gyrus, a region in the parietal lobe responsible for cortical speech and language. The study also found that all groups showed significant positive correlation with activity in the bilateral inferior frontal gyrus and anterior cingulate gyrus, further highlighting their role in speech and language. Interestingly, the same group also displayed decreased brain activity in the right and left precentral gyrus, which controls motor movement, and in the right cerebellum. This contrasts with previous studies regarding adult Parkinson's patients.

However, this is likely due to the different onset times of PD versus CP (Bakhtiari et al, 2017).

**BIG:**

The LSVT BIG program has the goal of moving "BIG." Because PD patients will often lose their range and speed of mobility, the therapy that involves increasing the amplitude of movements will help to delay those physical symptoms. Exercises include large movements such as outstretched arms and fingers, exaggerated strides and reciting phrases while walking to engage more areas of the brain (Fig 4). These large movements have resulted in increased trunk rotation, improved balance and gait, faster limb movements and a generally better quality of life.

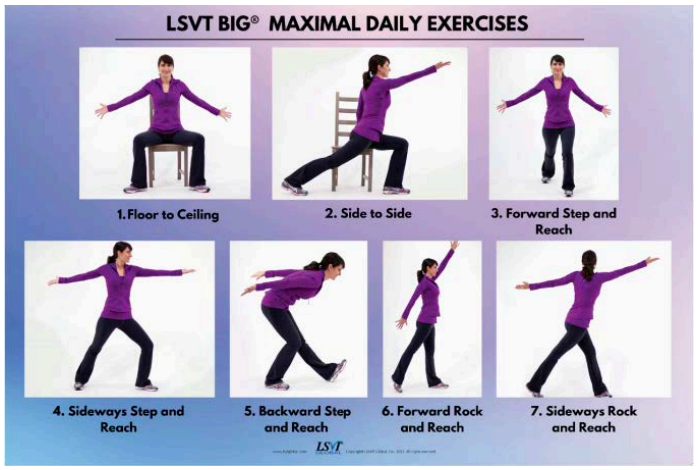


Figure 4: The exercises in the LSVT BIG's program emphasize the idea of moving "BIG" (LSVT Global)

In a study conducted with multiple PD patients, researchers looked at proprioceptive function, which measures body awareness and sensation, and found that the LSVT program led to significant improvements in these individuals. While difficult to discern the exact mechanism, one proposition is that sensory information acquired throughout the program is utilized in the basal ganglia. This subsequently causes rearrangement of dopaminergic pathways, allowing for sensorimotor improvement. This change was further confirmed by PET data. Alternatively, the newly acquired sensory information may be used on a cortical level to actually overcome sensorimotor function in the basal ganglia, which is similar to the neural

mechanism of those with amputation or stroke (Peterka, 2020).

There has been notably less research into the LSVT BIG program's physiological effects, primarily because of alternative treatments. Across the literature, it is well understood that exercise and repetitive movements are an effective way to manage PD symptoms. Exercise is known to increase the neuroplasticity of the brain. Neuroplasticity entails several processes, but it is simply the process by which the brain learns and solidifies new experiences and also modifies neural networks (Petzinger, G et al., 2013). Exercise acts on many aspects of neuroplasticity (i.e. releasing neurotrophic factors that improve cognitive function) which ultimately point to better outcomes. Additionally, in a recent study, there is also evidence to show that aerobic exercise also increases dopamine release in the anterior striatal region, possibly slowing the development of Parkinson's (Johansson, 2022).

## CONCLUSION

It is clear that the LSVT programs have proven to be effective in rewiring the brain and improving outcomes for Parkinson's patients. Still, there remain several criticisms of the program. A major concern is the long-term use of such an intensive program. Typically the LSVT exercises are taught to patients by a certified physical or occupational therapist. The program also requires daily maintenance, so there is a need for strategies that will assist patients in implementing these exercises outside of therapy sessions. The scheduling of the program is also quite time intensive as many clinics recommend three to four sessions per week. For many working or older adults, this is simply not feasible and would require modifications by a therapist or other professional (Clark, 2013).

Additionally, the initial research done on the LSVT program was completed by its program developers. It will be beneficial to have independent studies that validate its promises. While it is beyond the scope of this specific program, it is important to acknowledge that there remains a large population that cannot access these services if they live in certain regions, cannot afford therapy or do not have consistent internet access.

With Parkinson's disease still the second leading neurodegenerative disease among adults, it is a continuing public health concern. Through more education and increased awareness of these programs, its benefits can be delivered to many more individuals. With the advent of artificial intelligence and virtual learning, the programs are potentially revolutionary for those who live alone, in rural areas or without access to therapeutic care. As Western medicine works to combine pharmaceutical treatment with non-invasive medicine, the LSVT poses great potential to be a part of treatment programs for various neurodegenerative disorders.

## REFERENCES


1. American Association of Neurological Surgeons. (2024).
Parkinson's Disease. AANS.

2. Bakhtiari, R., Cummine, J., Reed, A., Fox, C. M., Chouinard, B., Cribben, I., & Boliek, C. A. (2017). Changes in brain activity following intensive voice treatment in children with cerebral palsy. Human brain mapping, 38(9), 4413–4429.

3. Burciu, R. G., & Vaillancourt, D. E. (2018). Imaging of Motor Cortex Physiology in Parkinson's Disease.
Movement Disorders, 33(11), 1688–1699.

4. Clark, H. M. (2013-2014).
Effectiveness of the Lee Silverman Voice Treatment (LSVT) in improving communication in individuals with Parkinson's disease: A critical review.
Western University.

5. Clark, J.fox, Cl, M., & Sc. (n.d.).

6. Cleveland Clinic. (2022, April 15). Parkinsonism: What It Is, Causes & Types. Cleveland Clinic.

7. Chen, R., Berardelli, A., Bhattacharya, A., Bologna, M., Chen, K. S., Fasano, A., Helmich, R. C., Hutchison, W. D., Kamble, N., Kühn, A. A., Macerollo, A., Neumann, W. J., Pal, P. K., Paparella, G., Suppa, A., & Udupa, K. (2022).
Clinical neurophysiology of Parkinson's disease and parkinsonism.
Clinical neurophysiology practice, 7, 201–227.

8. Dementia with Lewy Bodies and Parkinson Disease Dementia - Neurologic Disorders. (n.d.). Merck Manuals Professional Edition.

9. Fox, C. M., Morrison, C. E., Ramig, L. O., & Sapir, S. (2002). Current Perspectives on the Lee Silverman Voice Treatment (LSVT) for Individuals With Idiopathic Parkinson Disease. American Journal of Speech-Language Pathology, 11(2), 111–123.

10. Howells, D. W., Porritt, M. J., Wong, J. Y., Batchelor, P. E., Kalnins, R., Hughes, A. J., & Donnan, G. A. (2000). Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. Experimental neurology, 166(1), 127–135.

11. Johansson, M. E., Cameron, I. G. M., Van der Kolk, N. M., de Vries, N. M., Klimars, E., Toni, I., Bloem, B. R., & Helmich, R. C. (2022). Aerobic Exercise Alters Brain Function and Structure in Parkinson's Disease: A Randomized Controlled Trial. Annals of neurology, 91(2), 203–216.

12. Johns Hopkins Medicine. (2019). Can Environmental Toxins Cause Parkinson's Disease? Johns Hopkins Medicine.

13. Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis (T. B. Stoker & J. C. Greenland, Eds.). PubMed; Codon Publications.

14. Locus Coeruleus - an overview | ScienceDirect Topics. (n.d.).

15. Narayana, S., Fox, P. T., Zhang, W., Franklin, C., Robin, D. A., Vogel, D., & Ramig, L. O. (2010). Neural correlates of efficacy of voice therapy in Parkinson's disease identified by performance-correlation analysis. Human brain mapping, 31(2), 222–236.

16. NHS. (2022, November 3). Causes - Parkinson's Disease. NHS; NHS.

17. Parkinson's Disease Symptoms, Causes & Parkinsonism | MNC. (2019, November 27). Miamineurosciencecenter.com.

18. Parkinson's Foundation. (2024). Statistics. Parkinson's Foundation.

19. Peterka, M., Odorfer, T., Schwab, M., Volkmann, J., & Zeller, D. (2020). LSVT-BIG therapy in Parkinson's disease: physiological evidence for proprioceptive recalibration. BMC Neurology, 20(1).

20. Petzinger, G. M., Fisher, B. E., McEwen, S., Beeler, J. A., Walsh, J. P., & Jakowec, M. W. (2013). Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. The Lancet. Neurology, 12(7), 716–726.

21. Right-Shift Theory - an overview | ScienceDirect Topics. (n.d.).

22. Saffarian, A., Amiri Shavaki, Y., Shahidi, G. A., Hadavi, S., & Jafari, Z. (2019). Lee Silverman voice treatment (LSVT) mitigates voice difficulties in mild Parkinson's disease. Medical Journal of the Islamic Republic of Iran, 33(5), 5.

23. Sonne, J., & Beato, M. R. (2022, October 24). Neuroanatomy, Substantia Nigra. Nih.gov; StatPearls Publishing.

24. Stoker, T. B., & Greenland, J. C. (Eds.). (2018). Parkinson's Disease: Pathogenesis and Clinical Aspects. Codon Publications.

25. Weintraub, D., Aarsland, D., Chaudhuri, K. R., Dobkin, R. D., Leentjens, A. F., Rodriguez-Violante, M., & Schrag, A. (2022). The neuropsychiatry of Parkinson's disease: advances and challenges. The Lancet. Neurology, 21(1), 89–102.

# THE EMOTIONAL PROCESSING OF DREAMING AND DREAM ANALYSIS IN EXPLORING AND RESOLVING EMOTIONAL CONFLICTS

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**ABSTRACT**
This article explores how dreams and dream analysis contribute to emotional regulation and psychological development. Drawing from both neuroscience and psychoanalysis, the study considers how dreams may process traumatic and emotional material during REM sleep and how their interpretation in therapy can promote self-awareness and behavioral change. Two complementary frameworks are used: a connectionist model of emotional processing, which explains how dreams link emotionally similar memories to reduce psychological distress, and a cognitive-experiential model of dream analysis, which outlines a three-stage therapeutic approach—exploration, insight, and action. Together, these models highlight the interdisciplinary significance of dreaming as both a biological process and a psychotherapeutic tool.

## INTRODUCTION

Often when people think about dreams, the immediate association is with a fantasy created inside the mind. But going beyond that, dreams can be defined as a kind of mental activity that takes place during sleep and mostly consists of visual content perceived as vivid and hallucinatory, often with bizarre elements or irrational narratives (Cheniaux, 2006). These experiences are also connected to how the unconscious mind continues to function during sleep. Dreams can be interpreted and analyzed in relation to what is happening in a person's life and, through an understanding of psychoanalysis, can help individuals better understand themselves and, in doing so, achieve psychical growth. Consideration can also be given to the meanings and functions of dreams and how those aspects are related to each other.

With this in mind, several questions may arise that can be better addressed with insights from psychoanalytical and neuroscientific perspectives. These questions include: What are the reasons dreams occur? Is there a physiological reason for dreaming? How can dreams provide meaningful insights into someone's psychological condition and assist in treating that condition? How can neuroscience and physiological perspectives help explain the purpose of dreams?

To address such questions, the theory that dreams can be evolutionary adaptations is considered—adaptations that involve processes tied to survival, memory, and trauma. A deeper focus is placed on the processing of traumatic or emotional material within this theory. By converging those ideas, it becomes possible to explore how dreams and their interpretation might mitigate disruptive emotions. This approach makes it possible to examine how the emotional processing function of dreaming and the analysis of dream content can help reduce distressing feelings. Two models are brought together in this context: a physiological model supported by emotion processing theory, and a cognitive-experiential model of dream analysis. The first model examines the emotional processing function of dreaming through a psychological framework of connection nets. This model proposes that dreams make it easier for memories to connect with one another, which allows traumatic memories to be dissolved and emotional discomfort to be alleviated. The second model centers on dream analysis using a cognitive-experiential approach that includes three main discussion stages: exploration, insight, and action. These are the steps a therapist and patient go through to connect dream content with the patient's emotional conflicts. Through this process, the dreamer becomes more aware of the underlying schemas contributing to those conflicts, helping ease emotional distress and support personal development through behavioral change.

## INTERDISCIPLINARY FOUNDATIONS OF DREAM STUDY

To develop a more complex understanding of dreams, it is essential to consider the fields of psychoanalytical study, dream analysis, and neuroscience. Psychoanalysis explores how the unconscious and conscious mind contribute to diagnosing and treating mental conditions, often using dreams as a pathway to explore unconscious material. Dream analysis involves a collaborative process between patient and therapist in which dream content is explored to uncover issues connected to emotional struggles. The goal of this process is to generate insight, which may lead to psychological change.

A complete understanding of dreams and their functions must also include biological and neurological activity as examined through neuroscience. Neuroscience explores the chemical and biological functions of the brain at a molecular level, including the nervous system, brain, spinal cord, and peripheral system. Tools such as EEGs can reveal electrophysiological properties of dreaming, while neural net models may compare brain activity during wakefulness and dreaming. These tools support theories such as emotional processing during dreaming, where emotional experiences, particularly traumatic ones, are diffused through memory reconsolidation during sleep.

To address how dreams and their interpretation can alleviate emotional stress, two models are considered: the "connectionist nets" model and a cognitive-experiential model of dream analysis. The connectionist model demonstrates how neural links are distributed

widely, facilitating easier connection among memories. This idea is grounded in the theory of emotional processing. Meanwhile, the cognitive-experiential model describes dream interpretation as a process involving exploration, insight, and action. This model views dreams as personally meaningful; by understanding the schemas within dream images through elaboration and association, psychological development may occur.

## NEUROLOGICAL BASIS OF DREAMING

Before diving into the emotional processing aspect of dreams, their interpretations, and how they collaborate in solving emotional conflicts, it is important to understand the processes involved in the formation of dreams in the brain, when they occur, and other possible cognitive and emotional functions they might serve. It has also been scientifically observed that dreams are constructed through internally generated sensory, cognitive, and emotional experiences, primarily during REM sleep (Desseilles et al., 2011). REM sleep is the condition in which rapid eye movements occur and cortical blood flow—that is, the delivery of blood to the outermost layer of the brain—is very intense (Cheniaux, 2006). Cerebral blood flow is essential for eliminating neural waste and for delivering nutrients and oxygen to the brain (Tsai et al., 2021). It has also been demonstrated through positron-emission tomographic studies (PET scans) that during REM sleep, the associative visual cortex and the limbic and paralimbic regions are active, while the primary visual cortex and the prefrontal cortex are deactivated.

In the context of dream analysis by psychiatrists, these findings have been used to explain specific characteristics of dream content. Psychiatrists can connect the richness of visual imagery to the activated associative visual cortex and the deactivated primary visual cortex; the strong emotional response to the activation of the limbic and paralimbic regions; and, finally, the bizarreness, incoherence, loss of criticism, and forgetfulness to the deactivation of the prefrontal cortex. In addition to these neurophysiological aspects of dreaming, other possible purposes of dreaming beyond emotional conflict resolution have also been investigated. These include mental activity, threat simulation, wish fulfillment, and a variety of other proposed functions such as the discharge of psychical energy, problem solving (both intellectual and emotional), creativity, self-knowledge, integration of the mind, adaptation, learning, stress neutralization, and communication.

## THE EMOTIONAL PROCESSING FUNCTION OF DREAMING

After understanding how dreams occur in the brain, when they happen, and why, attention can now shift to the first half of this study: the emotional processing function of dreaming in resolving emotional conflicts, viewed through the aforementioned psychological connectionist nets model. According to psychiatrist, psychoanalyst, and associate professor Elie Cheniaux, this function relates to an elaboration process described in a model of computational neuroscience, which recognizes dreams as relevant to processing trauma and psychological conflict.

First, let's consider the dream's function of processing emotions and how this corresponds to the idea that it can alleviate stress. Emotional aspects are encoded as implicit memories (Cheniaux, 2006). Implicit memory implies that remembering can be an unconscious process (Martin & Li, 2016). Those implicit memories are likely consolidated during REM sleep, which is also when dreams occur the most. Scholars have agreed about there being a relationship between emotions that are felt in waking moments and the content that appears in our dreams (Cheniaux, 2006). The same study claims that, since REM sleep has been shown to collaborate in the processing of emotional memories, it is safe to conclude that emotions have a big impact when dreams are formed. This also allows us to understand that those mental activities have a therapeutic role as they process traumatic experiences and conflicts, just as sessions of therapy would.

To further comprehend this elaboration process, a model of computational neuroscience can be used. This process relies on the frequent neural network connections that occur during dreaming, which associate traumatic memories with other memories that have the same affective connotation, making those traumas less distressing and powerful. In other words, researchers argue that

these neural connections occur more easily during dreaming than during wakefulness (Cheniaux, 2006). This would, therefore, allow more connections and more elaborations to take place. Additionally, these neural networks are organized, as previously described, according to the emotions being experienced, which means that mental connections are grouped together based on affective similarity and tone. As a result, during sleep, more recent events are connected to past and more remote ones that share emotional relatedness before being stored as memories in the brain. This suggests that unpleasant situations—whether traumatic experiences or less disturbing, stressful memories—can be more easily associated and connected with other encoded memories while dreaming. These new connections would lead to fewer disturbing and unpleasant emotions being tied to those powerful and traumatic memories.

Another author uses the same model of computational neuroscience to argue that dreams serve the function of processing and mitigating negative emotions. Clinical psychologist Matthew Merced supports the idea that dreams have the ability to dilute emotional intensity, granting them a partial therapeutic role. He explains that dreams continuously add new memories to older, traumatic ones, resulting in the diffusion of the emotional intensity of those powerful memories. In comparison to the previously discussed scholar, Cheniaux, Merced explores in greater depth the ideas of one of the more specialized psychiatrists in the field of dream function. This allows for a clearer explanation of how the connection between remote and newer memories might occur. Merced examines Ernest Hartmann's studies on dreams, including his methodology, analyses, and conclusions.

Hartmann, a renowned psychiatrist, psychoanalyst, and sleep researcher, conducted a series of dream reports over several months with individuals who had experienced traumatic events. A recurring pattern was observed in which natural disasters appeared as common dream content. Over time, Hartmann recorded that the anguish linked to these nightmares diminished as they became associated with older, previously encoded material. Merced argues that Hartmann noticed a gradual decrease in the negative emotions connected to the traumatic experience and that the trauma began to have a smaller presence in the patient's waking life and dreams (Merced, 2012). Merced places more emphasis on the idea that these connections occurred between traumatic and older memories, rather than with newly encoded content. This difference could be due to Cheniaux referencing earlier interpretations of Hartmann's work. In addition, Merced includes the idea that the trauma's role becomes diminished in both dreams and waking life, offering further insight into how emotional resolution and integration may occur through the dreaming process.

Another author who supports the emotional processing function of dreams is Matthew Walker, a professor of psychology and neuroscience at the University of California, Berkeley, and director of the university's Center for Human Sleep Science. Walker states that dreaming can help reduce negative emotions and traumatic experiences because, during REM sleep, the brain is free from an anxiety-triggering molecule and the emotional and memory-related regions of the brain are activated. He also claims that while dreaming, the brain becomes more creatively active, mixing and combining memories in abstract and novel ways that help solve problems that may be unsolvable in waking life (Walker, 2017). This idea of connecting old and new memories to solve unresolved problems is closely related to the arguments presented by Cheniaux and Merced within the computational neuroscience framework. Creativity, in this context, would enable the brain to combine memories in ways that help resolve internal emotional "puzzles."

This aligns with the emotional processing theory, where creativity plays a role in mitigating strong and distressing emotions. These insights also introduce new elements to the theory of dream function—such as the brain's freedom from anxiety-inducing molecules during REM sleep and the active participation of brain regions involved in memory and emotion. In addition, the creative capacity of the brain to join and reconfigure memories and emotional material adds a significant perspective not emphasized by the previous two scholars who contributed to the emotional processing theory of dreams.

## COGNITIVE-EXPERIMENTAL DREAM ANALYSIS AND ITS THERAPEUTIC POTENTIAL

The second half of this study deals with the argument that working with dreams within the field of psychotherapy—specifically, through a cognitive-experiential model of dream analysis—can provide positive psychological development for the patient. In support of this, Merced also argues that dreams are events full of meaning, and that dream work, which assumes dreams are part of a cognitive process and have personal relevance, is a well-founded and therapeutically relevant activity. He claims that for dream work to be done, the psychologist must clarify and comprehend the images in the dreams through the dreamer's elaborations, thoughts, feelings, and associations. By understanding the visual content of the dream, both the patient and the psychoanalyst are better able to visualize previously hidden schemas, and from that, the possibility of positive change can emerge.

Merced adds that the simple act of talking about dreams also has therapeutic effects (Merced, 2012). He defends a three-step model developed by Clara E. Hill, a professor of psychology at the University of Maryland. The first step is exploration, the second is insight, and the third is action. Merced notes that these three stages mirror the structure of therapy more broadly. First, the therapist and the patient explore possible issues that brought the patient into treatment. Then, the therapist tries to make sense of the patient's underlying personal schemas and how they relate to emotional conflicts. Finally, both attempt to initiate change in the client's life.

Merced states that during the exploration stage of dream analysis, there is often an initial sense of not knowing exactly what the images in the dream mean. However, with open-ended questions the therapist can ask about the dream's visual content, the client can more easily connect the images to personal life experiences through free associations. With the help of the therapist, it becomes possible to arrive at current conflicts or situations in the patient's life that may relate to the dream's content. In the second stage, both participants work to combine the descriptions and associations in order to gather possible interpretations of the dream. In this stage, as in the first, the therapist asks the patient for initial impressions, and they both try to form interpretations that fit the individual's past and current history. The therapist should avoid relying on fixed or stereotypical meanings; interpretations should be personal. Again, the bizarre and figurative aspects of dreams are connected to the specific areas of the brain that are either activated or deactivated during REM sleep.

The third and final stage allows the patient to decide whether to change certain behaviors or reconsider perspectives after gaining insight into the dream's meaning. This may lead to psychological development, considering that behaviors and thoughts can change over time. If the client decides to make a change, the therapist can offer strategies the patient may follow. However, it is important to recognize that behavioral change is not required. Some patients may be more resistant to change than others; nevertheless, all responses are valid in the context of dream work. Simply working with the dream presented by the individual can already increase the dreamer's awareness of psychological issues, schemas, and personality dynamics. The therapist can also help evaluate the benefits and drawbacks of specific choices and offer encouragement (Merced, 2012). In this way, the patient is given tools to achieve personal development and ease emotional conflict.

Other authors agree with the view that dream work can contribute to psychological development and hold therapeutic value. In one article, Hill's cognitive-experiential model of dream analysis, along with other dream work frameworks, is used to support the argument that dream interpretation can promote cognitive restructuring and emotional benefit. These authors emphasize that the model focuses on building a shared understanding between therapist and patient based on the patient's associations and descriptions of the dream, with the aim of identifying personally meaningful insights and increasing self-awareness (Scarpelli, 2022). These scholars also note that, while the effectiveness of dream work in psychotherapy has not yet been definitively established, many models—such as Hill's—show consistency with experimental findings in the study of dreams. This is partly

because they avoid stereotypical interpretations and instead emphasize the individual's cognitive, emotional, and behavioral experience (Scarpelli, 2022).

**CONCLUSION**

In conclusion, dreams and dream analysis can have powerful consequences in cognitive, emotional, and behavioral functions. Dreams have an emotional processing ability that allows traumatic events or simply stressful emotions to have their intensity decreased so that the brain can work less disruptively. For this, the model of computational neuroscience helps demonstrate how, during dreaming, new connections are more easily made, allowing negative emotions to become less intense, less worrying, and less influential in the mind. In addition, the cognitive experimental model of dream analysis also shows potential benefits for psychological growth and therapeutic value by connecting the visual aspects of dreams with the dreamer's associations and descriptions in order to identify underlying conflicts. Through this process, psychological development can be achieved by fostering greater awareness of the self. In this way, both dreams and dream analysis can lead to personal growth, particularly through the cognitive development that results from these processes.

**REFERENCES**

1. Cheniaux, Elie. "Os Sonhos: Integrando as Visões Psicanalítica E Neurocientífica." *Revista De Psiquiatria Do Rio Grande Do Sul*, vol. 28, no. 2, 2006. DOI: 10.1590/s0101-81082006000200009.

2. Desseilles, Martin, et al. "Cognitive and Emotional Processes during Dreaming: A Neuroimaging View." *Consciousness and Cognition*, vol. 20, no. 4, 2011. DOI: 10.1016/j.concog.2010.10.005.

3. Martin, Jane, and Clara Li. "Normal Cognitive Aging." *Brocklehursts Textbook of Geriatric Medicine and Gerontology*, Section D, Elsevier, 2016. DOI: 10.4324/9780203882504-7.

4. Merced, Matthew. "Dreaming: Physiological Sources, Biological Functions, Psychological Implications." *The Journal of Mind and Behavior*, vol. 33, no. 3/4, 2012.

5. Scarpelli, Serena, et al. "What about Dreams? State of the Art and Open Questions." *Journal of Sleep Research*, vol. 31, no. 4, 2022. DOI: 10.1111/jsr.13609.

6. Tsai, Chia-Jung, et al. "Cerebral Capillary Blood Flow Upsurge during REM Sleep Is Mediated by A2A Receptors." *Cell Reports*, vol. 36, no. 7, 2021. DOI: 10.1016/j.celrep.2021.109558.

7. Walker, Matthew. "Why Your Brain Needs to Dream." *Greater Good*, 24 Oct. 2017.

# THE HIDDEN WITNESSES TO MURDERS: AN INTRODUCTION TO FORENSIC ENTOMOLOGY

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**ABSTRACT**
Forensic entomology, the study of insects in legal investigations, plays a crucial role in determining the postmortem interval (PMI) and other factors that can help determine the time since death. For instance, forensic entomology has applications in identifying toxicological influences and helping reconstruct potential crime scenes. This article aims to provide an overview of forensic entomology in the context of how it is used to determine the postmortem interval, providing a general overview of the techniques used—such as successional waves and entomotoxicology—and highlighting its significance in its field. Furthermore, future implications and interdisciplinary collaborations will be explored, redefining the field with new technological innovation. However, potentially concerning impacts from climate change may arise as a result. By highlighting both the present and future, this article aims to showcase the nature of forensic entomology in modern criminal investigations as a valuable tool to bring justice to victims of violent crime.

## SECTION 1: THE INTRODUCTION

### WHAT IS FORENSIC ENTOMOLOGY?

Forensic entomology is considered the study of insects and arthropods in criminal investigations (Joseph, Mathew, Sathyan, & Vargheese, 2011). Often, a decomposing body may be past the point of when traditional techniques, such as rigor mortis (or body stiffness), liver mortis (the settling of blood in the lower parts of the body after death) can be used. These techniques may only work past a certain point after death, usually within 3-72 hours after death (Bucholtz, 2024; Eden & Thomas, 2025; Shrestha, Kanchan, & Krishan, 2025). After this point, insects that colonize the body in search of food and warmth may be used to estimate the time of death. Forensic entomologists may study the changes in insect population and developing larvae to estimate the postmortem interval (PMI), which is the time interval between physiological death and the examination of the deceased person (Munro & Munro, 2008).

### IMPORTANCE OF FORENSIC ENTOMOLOGY

Deceased bodies can be thought of as mini ecosystems with insects and other microorganisms interacting with the abiotic, "deceased-body" environment. Because of these interactions, insects reproduce on the body and use the abundance of resources offered by a deceased body—such as warmth, food, and space. Given the complexity of crime scenes, forensic scientists try to answer the six W's: why, who, where, how and what. Insects can provide a whole host of clues that can lead to answers to these questions (Viero, Montisci, Pelletti, & Vanin, 2019). For instance, they can help identify any evidence of abuse and/or neglect through the presence of maggots in open wounds and unclean areas or on clothing. Of note, the higher the number of maggots potentially found in wounds, the higher the likelihood of abuse/violence prior to death (Anderson, n.d.). Additionally, they may provide vital information about the location of death. The presence of a certain species of insect could show where a body was found after death, due to specific environments known to support specific types of insects (Anderson, n.d.; Wyland & Wyland, 2024). Insect evidence collected from the body may be used to then formulate further clues. Another important contribution from insects is their role in identification through DNA analysis. A larvae's stomach contents can be analyzed to, first, identify the victim and, second, link a potential suspect to the crime scene. One study suggested that short tandem repeat (STR) analysis of a maggot's crop content can be used to associate maggots to crime scenes (Kondakci et al. 2019). STR analysis is a DNA profiling technique that compares the number of repeated sequences in DNA regions between samples, often involving secondary techniques (Barcelos et al., 2019). Combining this with further clues from the investigation may help direct detectives to the culprit or give an alibi to another suspect. For this reason, insects can be considered the tiny witnesses to crimes, and provide crucial evidence for catching the correct culprit.

## SECTION 2: THE TECHNIQUES OF FORENSIC ENTOMOLOGY

### THE USE OF INSECT LIFE CYCLES AND SUCCESSIONAL WAVES

Even though a multitude of different techniques are used for analysis, this article aims to shed light on some of the most common but crucial techniques that can help provide clues to investigators. Particularly, this article will emphasize two main techniques and their methods: Use of successional waves and insect life cycles and entomotoxicology and other chemical analyses.

It is also Important to note that every case is different: it is not necessary that investigators use the same techniques each time a body needs analysis due to the complex interplay of factors that may determine technique selection. For example, the stage of decomposition, presence of wounds, weather conditions etc can all influence what techniques will provide true evidence to point investigators in the right direction (Bambaradeniya, Magni, & Dadour, 2023).

Overall, this section will cover the methodology, applications, and limitations of the techniques listed above, and highlight their importance in uncovering crucial evidence that contributes to providing justice to victims.

As defined by the Oxford English Dictionary, the process of succession in ecology is "a process by which a plant or animal community

successively gives way to another until a stable climax is reached." In this context, when one insect colonizes a body, it interacts with that body (e.g. through its consumption, or use of a body's space as a breeding ground). As a result, it changes the corpse's conditions, which after an insect's death, becomes more suitable for another species to colonize. This cycle continues until a stable climax community is reached, a situation where one species may dominate, or several species may coexist. This technique is also related to the estimation of the postmortem interval and is applicable mostly to later stages of decomposition (Sharma, Garg, & Gaur, 2015).

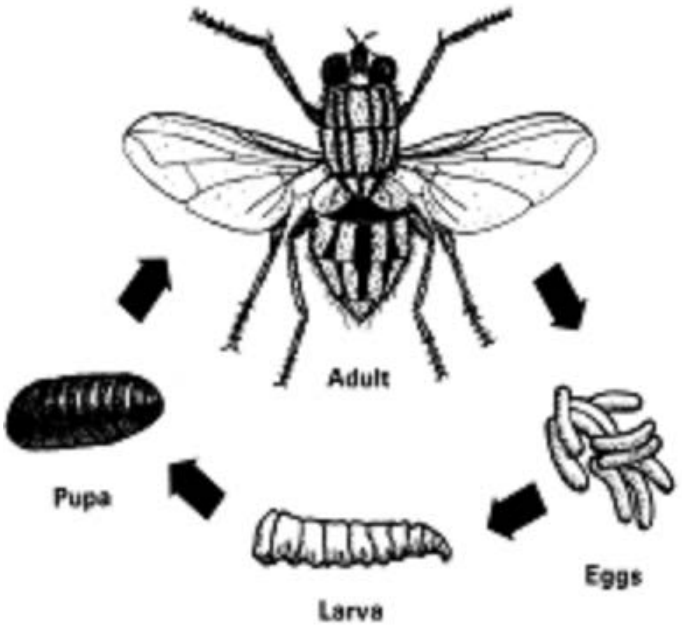



Figure 1: The life cycle of a fly (Illinois Department of Public Health, n.d.)

To use this technique, however, insect life cycles also need to be considered. For most insects, their life cycle consists of 4 stages: the egg, larva, pupa and adult. Typically, the greatest number of species will be attracted to a body during putrefaction, a stage of bloating that typically happens after days 2-7 of death. This leads to the first step of estimating the PMI: identifying the species of insect present. Next, the age of the larva can be estimated by measuring the length or dry weight of the oldest larva, comparing it with reference data found in a database. It is also important to note that the rate of development of a larva is dependent on the surrounding temperature. Thus, it is important to obtain a thermal history (the history of temperature changes for that environment), which can thus be compared to the temperatures at the death scene. The PMI can then be estimated by backtracking the life cycle of the identified species (Sherlock Institute for Forensic Science, n.d.). The equation for this can typically be expressed by Accumulated degree hours (ADH):

**ADH = (temperature at crime scene – base temperature) x time elapsed**

The base temperature corresponds to the minimum temperature at which the insect can develop, and time elapsed refers to the duration of each developmental stage at that temperature (Franceschetti et al., 2021).

Other factors also may affect insect activity—such as day length, oxygen levels and food quality. In terms of colonization sequence, flies arrive within a few hours of death, attracted to odors of decomposition. These flies are typically the most useful for estimating time of death. These are followed by carrion beetles, which typically arrive within a few days. Following this, carpet beetles, which consume the bone, hair and skin, may arrive during the dry stage of decomposition (North Carolina School of Science and Mathematics, 2013). Looking at this common colonization sequence, succession can be seen in action, as some insects prefer food sources more available during later stages of decay. These later stages are made possible through the interaction of earlier insect species, which make conditions more favorable for these later colonizers. As a result, using a combination of insect life cycles and cycles of succession can help estimate the time since death.

Another aspect to note is that obtaining live specimens on crime scenes requires no special training and minimal equipment, meaning that CSI personnel can learn to collect samples quickly and at a low cost (Volckaert, 2020). However, it is also important to note that environmental factors, such as humidity and temperature can affect the rate of development of insects, and thus the speed of successional waves, which may lead to an overestimate or underestimate of the time since death. Even though temperature is considered in the equation above, other factors, such as

humidity or wind, are not. These factors can also affect development, and therefore, successional waves.

## ENTOMOTOXICITY AND CHEMICAL ANALYSES

Entomotoxicology is a relatively new branch of forensic entomology, where insects are used to detect drugs and other toxins in decomposing tissues. It also investigates the effects caused by drugs and toxins on arthropod development. The use of this technique may be especially helpful for the recent increase in drug-related deaths, connected to poisoning or overdoses (Introna, Campobasso, & Goff, 2001).

The technique works by using techniques such as gas chromatography (GC) and thin layer chromatography (TLC), which can help separate and analyze the components of unidentified fluids. These methods involve the use of a stationary phase (a starting point for any substance, which doesn't move) and a mobile phase (often a gas or solvent that can move throughout a column and help substances separate through their density, or solubility in the mobile phase). Often, to incorporate this technique, samples of insects will be homogenized and dissolved in a solvent to then go through GC or TLC.

However, this is not the only way that chemical analysis can be used: drugs and toxins can also influence the rate of development of certain insects. For instance, one study found that, in toxin-contaminated tissues, maggot development accelerated 36 hours after hatching. This was specifically observed on toxin-contaminated liver or spleen tissue. This pattern is abnormal as compared to normal maggot growth (Goff, Omori, & Goodbrod, 1989).

Additionally, another study demonstrated how these analyses can lead to real clues. In this study, a woman was found deceased in the early putrefaction stage (the bloated, discolored stage that occurs due to the build up of several gasses after death). Maggots found on the face and upper torso measured about 7.5 mm on average while just a single maggot from the woman's nostrils measured 17.7 mm, suggesting accelerated growth that was dependent on cocaine. A subsequent investigation revealed that the woman was a cocaine abuser (Lord, 1990).

However, this technique does not only apply to the deceased. Chemical analyses can also be used to gather clues from cases of abuse or neglect. For instance, DNA analysis from the earlier blood feed of head lice can be used as markers for the length and frequency of abuse periods. One case found an elderly victim of neglect who had body lice present down to the torso, and the egg laying pattern suggested that the victim had suffered 2 years of continuous neglect (Lambiase & Perotti, 2019). Signs like these may help investigators protect the welfare of the elderly.

Additionally, pollen grains on corpses may also be analyzed as evidence. Even though it is usually thought of as a technique to identify climate change throughout history, by analyzing the pollen grains present (eg through identifying their shape, size and structure), and combining potential databases, pollen grains can be used to identify certain plant species (PaleoResearch Institute, n.d.). Thus, the original climate where the body may have initially been found can be identified. This is because different insects tend to prefer to inhabit different plants, and different plants grow better in some climates than others. This is especially helpful if the body is suspected to have been moved from its original place, again providing alibis to potential suspects, while connecting others to a particular location.

## DRAWBACKS OF TECHNIQUES USED IN FORENSIC ENTOMOLOGY

One of the biggest drawbacks is environmental variability. Most insects require extremely specific temperatures and humidity levels to breed. Therefore, environmental variability can cause disturbances to the distribution and behavior of fly larvae (Bansode et al., 2025). As a result, forensic investigations relying on insect evidence might be compromised due to unpredictable environmental conditions that can alter larval growth, timeline of development, and overall patterns of distribution.

Additionally, the presence of non-forensically relevant insects can also make establishing successional waves and back-tracing patterns extremely challenging. Most of the time, these

insects may be present on a corpse because they are attracted to a corpse to feed, as predators, on the necrophagous insects (the insects consume the rotting flesh of a given corpse) that are already present (Amendt, Richards, Campobasso, Zehner, & Hall, 2011). However, these patterns do not always occur simultaneously on a corpse. They can also be predicted chronologically, but 2 conflicting waves of succession may be harder to differentiate as the cadaver further decays.

Deceased bodies may also be found in an extremely wide range of conditions – some cases have been found in dumpsters, while others have been found in places such as water tanks (Elisa Lam case), or inside of a dinosaur statue (Jones, 2021). Due to each case having the potential to be extremely unique, there may not be any previous data (e.g. temperature ranges or humidity estimates) available for certain regions, as making estimates relies on data that is region-specific. As a result, estimates of the PMI may be challenging to make in certain cases.

## SECTION 3: FUTURE IMPLICATIONS AND CONCLUSIONS

### IMPACTS OF CLIMATE CHANGE

As mentioned previously, environmental factors, namely temperature, can have massive impacts on insect development rate and potential speed of successional waves. However, climate change is now shifting the global distribution patterns of necrophagous insects, potentially shifting species that are typically found near the equator north due to global warming. This could affect their ability to colonize cadavers at crime scenes, complicating the calculations of the estimations of PMI. However, climate change is also causing a general decline in insect populations due to higher use of pesticides, deforestation, global warming and pathogens, to name a few (Amendt, 2021).

Rising global temperatures may also lead to faster insect development, and therefore an underestimation of the PMI if standard models are used (Amendt, 2021). Climate change can also cause unpredictable weather patterns, potentially resulting in patterns that lead to colder winters and warmer summers. These more unpredictable patterns may lead to an inconsistency within models currently used to estimate PMI (Matuszewski, Szafałowicz, & Grzywacz, 2014).

### TECHNOLOGICAL ADVANCEMENTS AND INTERDISCIPLINARY COLLABORATIONS

There are now several advancements within the field, which now has an even greater potential for interdisciplinary collaboration. For instance, soil may contain enormous potential as an indicator for PMI because succession in microbes is vital to decomposers and is provided with ammonia rich fluids from the body (News-Medical, 2020).

Additionally, newer AI models may be trained on genomic information containing information on entire microbial communities. One method that has been used is machine learning. Specifically, a method called an artificial neural network (ANN), which involves developing algorithms to enable computers to learn from existing databases without explicit programming, has proved useful (Wang et al., 2022).

However, the most common type of machine learning algorithm being used for microbial community studies is the random forest (RF) regression model, which may be able to predict microbial succession patterns on a cadaver through capturing non-linear patterns in the several variables that may impact PMI estimations. This means that it could incorporate multiple variables such as species specific growth rates, humidity, and effects of drugs all at once (Belk et al., 2018).

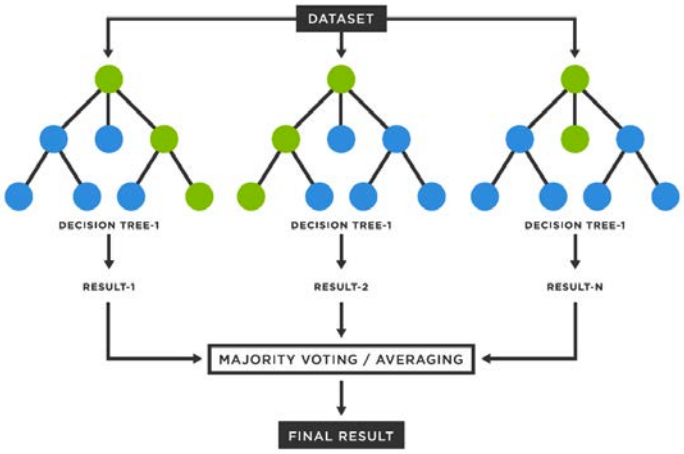



Figure 2: The mechanism behind the random forest model (Gunay, 2023)

Overall, the integration of RF models and microbiome analysis is a significant advantage over traditional regression models, offering greater accuracy and adaptability to diverse environments. However, it is important to note that these methods are still experimental and have not yet been officially approved as a standalone method for use in forensic entomology. Thus, further research may need to be conducted to validate their reliability.

## CONCLUSIONS

Overall, advancements in techniques like entomotoxicology, DNA barcoding, and machine learning models such as random forest (RF) are helping forensic entomologists improve the accuracy and precision of estimating PMI and other clues that can find the true culprit of a crime. Collaboration between experts is also shown to be vital to make strides in the field, and can involve the collaboration of entomologists, CSI personnel, and even computer scientists. Despite earlier limitations, like environmental variability (which can affect insect development), advancements in technology such as the development of machine learning models have significantly reduced errors and the effects of limitations by increasing accuracy and precision of analysis, making entomology a more reliable tool in the justice system.

Additionally, interdisciplinary collaborations have the potential to play a vital role in advancing these methodologies. These collaborations have the potential to be much more thorough tools for analysis. Ongoing research and collaboration are vital pillars to keep up with environmental challenges, such as climate change and unpredictable weather patterns. Moving forward, continued innovation and refinement of techniques will be essential parts of keeping forensic entomology a reliable tool in the justice system.

## REFERENCES


1. Amendt J. (2021). Insect Decline-A Forensic Issue?. *Insects, 12*(4), 324.

2. Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J. R. (2011). Forensic entomology: applications and limitations. *Continuing Medical Education Review, 7*(7), 379–392.

3. Bambaradeniya, T.B., Magni, P.A., & Dadour, I.R. (2023, June 14). Concepts, Procedures and Techniques by Forensic Entomologists. *Encyclopedia*.

4. Barcelos, D., Funabashi, K., Mazloum, S. et al. (2019). STR technique for the detection of contamination by exogenous DNA in paraffin blocks and histological slides. *Surgical and Experimental Pathology, 2*, 25.

5. Belk, A., Xu, Z. Z., Carter, D. O., Lynne, A., Bucheli, S., Knight, R., & Metcalf, J. L. (2018). Microbiome Data Accurately Predicts the Postmortem Interval Using Random Forest Regression Models. *Genes, 9*(2), 104.

6. Bucholtz, J. (2024, August 20). How to estimate time of death using algor Mortis, explained. *American Military University*.

7. Deniz Gunay. (2023, September 11). *Random Forest*. Medium.

8. Eden, R. E., & Thomas, B. (2023, August 28). Algor Mortis. In *StatPearls*. StatPearls Publishing.

9. FORENSIC ENTOMOLOGY: THE USE OF INSECTS. (n.d.).

10. Franceschetti, L., Pradelli, J., Tuccia, F., Giordani, G., Cattaneo, C., & Vanin, S. (2021). Comparison of Accumulated Degree-Days and Entomological Approaches in Post Mortem Interval Estimation. *Insects, 12*(3), 264.

11. Goff, M. L., Omori, A. I., & Goodbrod, J. R. (1989). Effect of cocaine in tissues on the rate of development of *Boettcherisca peregrina* (Diptera: Sarcophagidae). *Journal of Medical Entomology, 26*, 91–93.

12. Illinois Department of Public Health. (n.d.). *Public Health Pest Manual – Filth Flies*.

13. Introna, F., Campobasso, C. P., & Goff, M. L. (2001). Entomotoxicology. *Forensic Science International, 120*(1–2), 42–47.

14. Jones, S. (2021, May 25). Missing man found dead inside Spanish dinosaur statue. *The Guardian*.

15. Joseph, I., Mathew, D. G., Sathyan, P., & Vargheese, G. (2011). The use of insects in forensic investigations: An overview on the

scope of forensic entomology. *Journal of Forensic Dental Sciences, 3*(2), 89–91.

16. Kondakci, G. O., Bulbul, O., Shahzad, M. S., Polat, E., Cakan, H., Altuncul, H., & Filoglu, G. (2009). STR and SNP analysis of human DNA from *Lucilia sericata* larvae's gut contents. *Forensic Science International: Genetics Supplement Series, 2*(1), 178–179.

17. Lambiase, S., & Perotti, M. A. (2019). Using human head lice to unravel neglect and cause of death. *Parasitology, 146*(5), 678–684.

18. Lord, W. D. (1990). Case histories of the use of insects in investigations. In *Entomology and Death: A Procedural Guide*(pp. 9–37). Joyce's Print Shop.

19. Matuszewski, S., Szafałowicz, M., & Grzywacz, A. (2014). Temperature-dependent appearance of forensically useful flies on carcasses. *International Journal of Legal Medicine, 128*(6), 1013–1020.

20. Munro, R., & Munro, H. M. C. (2008). Estimation of Time since Death. In *Animal Abuse and Unlawful Killing* (pp. 88–93). W.B. Saunders.

21. News-Medical. (2020, March 11). Microbial Succession as a Forensic Tool: Estimating time of death.

22. North Carolina School of Science and Mathematics. (2013, March 25). Estimating Post Mortem Interval, part 5 – Forensic Entomology [Video]. *YouTube*.

23. Oxford English Dictionary, s.v. "succession (n.), sense IV.14.g," December 2024.

24. PaleoResearch Institute. Pollen Analysis.

25. Ruchi Sharma, Rakesh Kumar Garg, J.R. Gaur. (2015). Various methods for the estimation of the post mortem interval from Calliphoridae: A review. *Egyptian Journal of Forensic Sciences, 5*(1), 1–12.

26. Sarika Bansode, Abhay Morajkar, Vinod Ragade, Vitthal More, Kiran Kharat. (2025). Challenges and considerations in forensic entomology: A comprehensive review. *Journal of Forensic and Legal Medicine, 110*, 102831.

27. Sherlock Institute for Forensic Science. Stages of insect life cycle | Forensic Entomology.

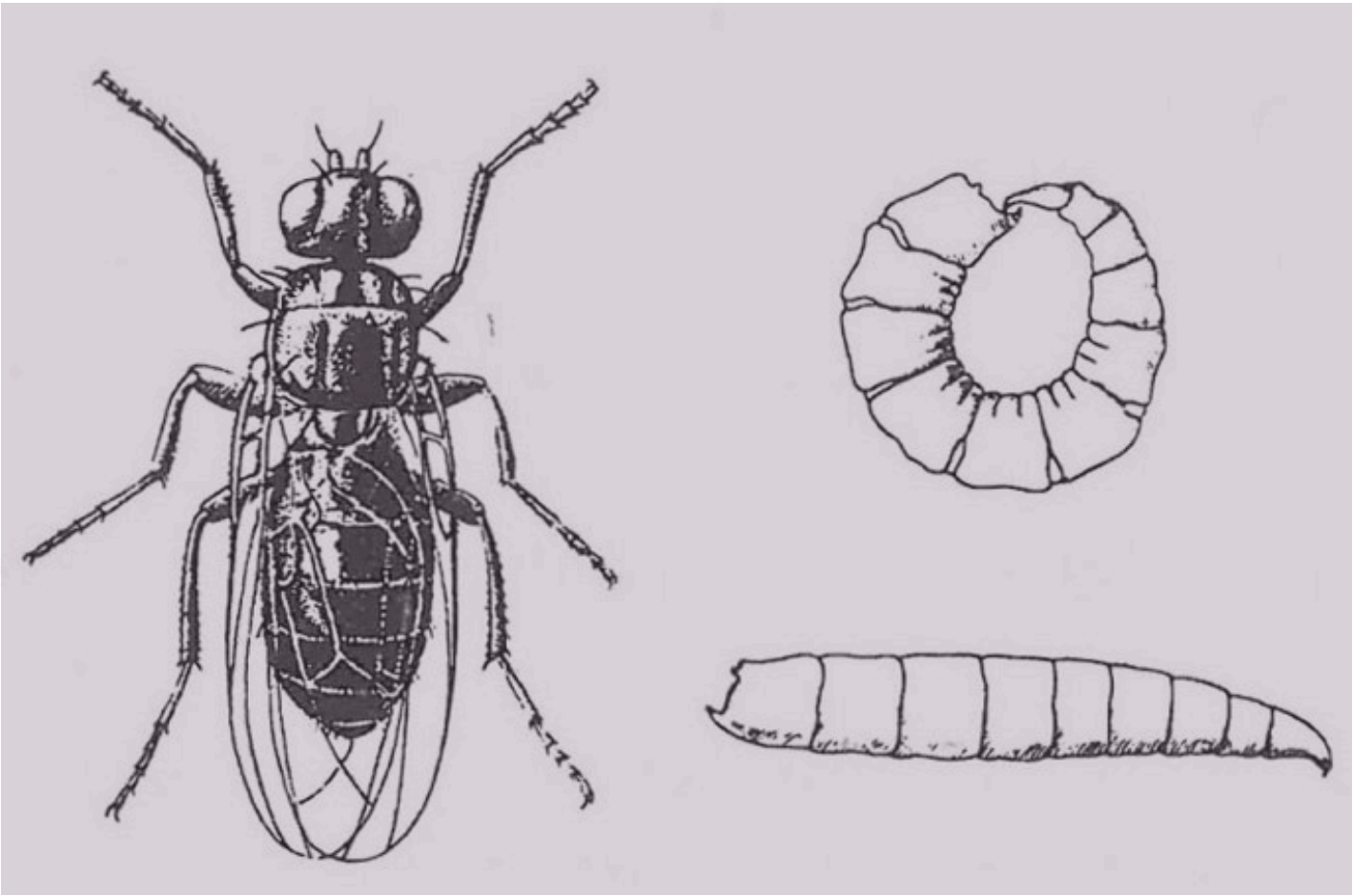
28. Shrestha, R., Kanchan, T., & Krishan, K. (2023, May 30). Methods of Estimation of Time Since Death. In *StatPearls*. StatPearls Publishing.

29. Viero, A., Montisci, M., Pelletti, G., & Vanin, S. (2019). Crime scene and body alterations caused by arthropods: implications in death investigation. *International Journal of Legal Medicine, 133*(1), 307–316.

30. Volckaert, H. (2020). Current Applications and Limitations of Forensic Entomology. *Themis: Research Journal of Justice Studies and Forensic Science, 8*(1), Article 4.

31. Wang, Z., Zhang, F., Wang, L., Yuan, H., Guan, D., & Zhao, R. (2022). Advances in artificial intelligence-based microbiome for PMI estimation. *Frontiers in Microbiology, 13*, 1034051.

32. Wyland, J., & Wyland, J. (2024, October 28). Forensic Entomology: How Insects Help Solve Crimes. *Entomology and Nematology Online Programs*



An illustration of the cheese skipper fly, *Piophila casei*, and its larval and pupal stages, often encountered in forensic investigations. *P. casei* typically colonizes decomposing remains during the later stages of decay, particularly when tissues are rich in fat and protein. Their presence can help estimate the postmortem interval (PMI), especially when earlier-arriving insects have completed their life cycle. Additionally, because *P. casei* thrives in warm, enclosed, or dry environments, their appearance may indicate that a body was stored indoors, moved postmortem, or decomposed in concealed conditions.

*Image credits: Pestium. (n.d.). The cheese skipper.*

# BEHAVIOR AND PERSONALITY: NATURE OR NURTURE?

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**ABSTRACT**
While children share only 50% of their genes with each parent, the behavioral and personality resemblances often appear too strong to be coincidental. This paper explores the interplay between genetic inheritance and environmental influence in shaping human behavior. Through foundational behavioral psychology studies, scientists have demonstrated that genes play a significant role in behavioral traits, with heritability estimates suggesting that roughly 40% of behavioral variation is attributable to genetics. These findings are supported by Genome-Wide Association Studies (GWAS), which have identified specific genes linked to the Big Five personality traits. The paper also addresses the ethical implications of genetic manipulation in behavior-related therapies. While environment undeniably shapes personality, the evidence points to genetic influence as a dominant force in behavioral development.

## INTRODUCTION

Imagine a father and son sleeping on their right sides with arms crossed across their chest, or a mother and daughter mirroring each other's short-temperedness. Only 50% of genes are shared between a parent and child, yet their behaviors match too well. Nature is full of moments like these that leave us questioning whether it is the genes or the observation and imitation of the parent that causes such a close resemblance in behavior and personality. In the world of behavioral psychology, the nature of genes and the nurture of the environment both play a role in a child's development, usually one more than the other. Even though we often only attribute genes to the expression of physical characters, genes also play a huge role in the passing of certain behavioral and personality traits from parent to child.

## TYPES OF BEHAVIORAL STUDIES

How did psychologists discover the underlying genetic influence of behavior? They simply conducted different types of studies where they strategically controlled for genetic or environmental variability. The most crucial studies that contributed to the modern understanding of behavioral psychology are twin and adoption studies, which follow the Equal Environments Assumption (EEA). According to the National Institute of Health, the EEA states that monozygotic (MZ) and dizygotic (DZ) twins experience nearly identical environmental conditions relevant to the trait of interest, allowing us to attribute any of those trait differences primarily to genetic differences. Twin studies observe the behaviors between MZ (identical) twins and DZ (fraternal) twins. In a typical family tree, two siblings share 50% of their genes.

However, MZ twins share a full 100% of their genes with each other. If they share 100% of their genes and they are under the same environmental conditions, we expect them to be 100% identical in their appearances and mannerisms. This means that any observed behavioral differences can be attributed to their environment. In contrast, DZ twins share only 50% of their genes with each other. Under the EEA, or assuming that the twins grow up under nearly identical environmental and familial conditions, any differences in behavior between the DZ twins can be largely attributed to that remaining 50% of genetic variation.

Figure 1 demonstrates the similarity between the reading abilities of two monozygotic twins. The slope displays a strong, positive linear correlation between the reading ability of one monozygotic twin and the reading ability of the other twin. When looking at the graph that reflects the reading abilities of two DZ twins, who only share 50% of their genes, more points can be observed scattered further away from the best-fit line, indicating only a moderate but still positive linear relationship between their reading abilities. Using these two graphs as evidence, a reasonable prediction is that sharing a larger percentage of genes produces a stronger similarity in behavior.

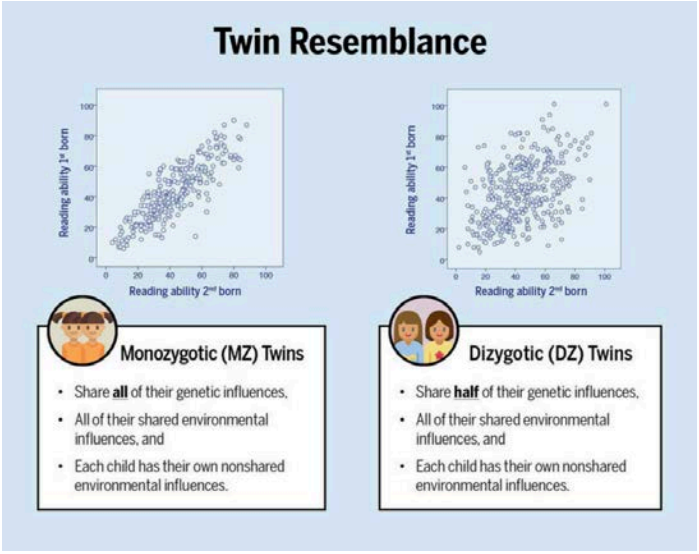


Figure 1: Two graphs showing the strength of concordance between the two twins, which is measured by the steepness of the slope of each graph. In other words, the slope of each graph represents the amount of similarity between the two twins' reading ability. The purpose of this comparison is to identify the role of genes in behavioral traits that are commonly believed to not have a strong correlation with genes (Hart, et al, 2021)

Adoption studies also played a huge role in understanding the genetic influence on behavior. They study behavioral similarities and the percentage of genes shared between a parent and their child, either biological or adopted. This type of study consists of the control group of a birth child and their birth parent. In this group, 50% of genes are shared,

and their environments are identical.

This model resembles that of genetic x environment interaction models, where it is given that environmental conditions are kept consistent to eliminate the influence of a variable environment on behavior. According to such genetic x environment interaction models, most of their behavioral differences can be attributed to their difference in genes. The first experimental group consists of an adopted child and their biological parent. In this group, 50% of their genes are shared, but their environments are different. Thus any behavioral similarities between the adopted child and biological parents are still attributed to genes. The second and final experimental group consists of an adopted child with their adoptive parent. In this case, a very low percentage of their genes are shared, but their environments are identical. As such, any behavioral similarities between the two can be attributed to their environment. A study conducted by Myers and Dewall explored the child-parent correlation in verbal abilities scores, aiming to discover to what extent genetics and environment play a role in a seemingly inheritable trait.

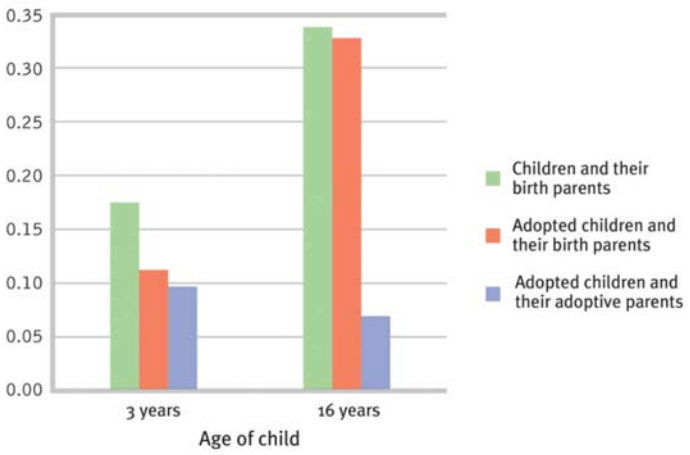


Figure 2: Data from Plomin and DeFries (1998) correlations between child and parents on verbal ability (Myers and Dewall, 2018)

In Figure 2, the graph aims to illustrate the strength of genetic influence compared to environmental influence by observing changes in a child's verbal ability score. According to the graph, the percentage of behavioral similarity between a child (whether birth or adopted) and their biological parents surpasses that between an adopted child and adoptive parents. These two groups, with an overwhelmingly high proportion of behavioral similarities, are the same ones that attributed behavioral similarities to genes, which then supports the claim that genes do indeed play a large role in the behavior of a child. However, that is not to say that the environment does not play a role at all; environmental factors will always have some influence on the similarities between a parent and child, yet statistically, genetic influence is stronger.

After examining the results of twin and adoption studies, scientists found that approximately 40% of behavioral differences can be attributed to genes. In other words, 60% of behavioral similarities can be attributed to genes. Within the twin studies specifically, researchers controlled gene-environment interactions, adjusting environmental factors between two individuals with similar genes to differentiate between genetically and environmentally influenced behaviors. This degree of caution leads into the concept of heritability.

**HERITABILITY**

Revisiting twin studies, heritability measures how much of the variability between individuals is really due to a difference in genes. In simpler terms, heritability measures the genetic degree of similarity between two individuals.

A high heritability means that differences in behavior are not explained by differences in genes. On the flip side, a low heritability means that the differences in behavior are better explained by differences in genes. Following this idea, monozygotic twins have a high heritability. Since they share close to 100% of their genes with each other, their behaviors are expected to be identical.

As such, their behavioral differences are better explained by environmental rather than genetic factors. On the contrary, dizygotic twins only share 50% of their genes with each other, so they have a low heritability, meaning their behavioral differences are better explained by that 50% difference in genes (Campos, 2019).

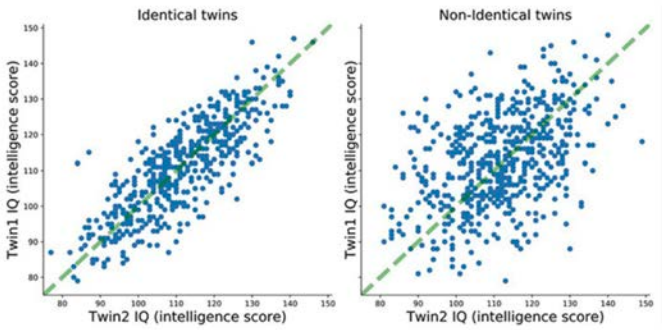


Figure 3: Two scatterplots that aim to identify the heritability of IQ by comparing similarities between monozygotic (identical) and dizygotic (fraternal) twins (Campos, 2019)

The graph measuring the IQ similarity between dizygotic twins does not have an obvious linear relationship, and the data points appear more random, thus indicating that there is a low correlation in IQ between twin 1 and twin 2. However, looking at the IQ scatterplot comparing monozygotic twins, we see a strong positive linear relationship between twin 1 and 2, indicating that there is a strong correlation in IQ scores between monozygotic twins. Thus, as these types of studies have discovered, we can conclude that IQ has a high heritability.

Beyond familial studies and the concept of heritability, genome-wide association studies tackle the cellular level of genetic influence on behavior and personality traits.

## GENOME-WIDE ASSOCIATION STUDIES AND THE BIG 5

Genome-wide association studies (GWAS) intentionally search for single nucleotide variations within the same type of genes among different individuals that contributed to a particular trait, which includes those of behavior and personality. To be more exact, genes directly influence cognitive traits (mental capabilities of the brain), which in turn influence the behavior and actions of the person. GWAS has been used to identify specific genes that influence the Big Five personality factor model that covers neuroticism, extraversion, agreeableness, conscientiousness, and openness to experience (Lo, 2017).

The first trait, neuroticism, refers to people with emotional instability, psychological distress, low self-esteem, and negative emotions. The genes associated with this trait are MAGI1 and variants along the 8p23.1 chromosome and in the L3MBTL2 gene (Vinkhuyzen, 2012). The second trait, extraversion, refers to a person's inclination to involve themselves in social circles and positive emotions.

Surprisingly, extraversion correlates with ADHD, meaning that certain genes that cause ADHD also contribute to the personality trait of extraversion. Some of these genes include variants along the WSCD2 and near PCDH15 genes (Vinkhuyzen, 2012). The third is agreeableness, which mirrors a person's cooperativeness and compassion for others. The fourth is conscientiousness, or in other words, order and discipline. The KATNAL2 gene in particular contributes heavily to this trait (Lo, 2017).

Lastly, openness to experience implies intellectual curiosity and creativity, which in turn is also associated with schizophrenia and bipolar disorder. As such, some of their common genes include RASA2 and PTPRD (Lo, 2017). Figures 4 through 8 provide a visualization of which chromosomes each Big Five personality trait is most associated with (Lo, 2016).

The big five personality traits encompass multiple smaller traits we often associate with others and share a fine line with certain disorders, yet that only supports the argument that behavior and personality are heavily influenced by genes.

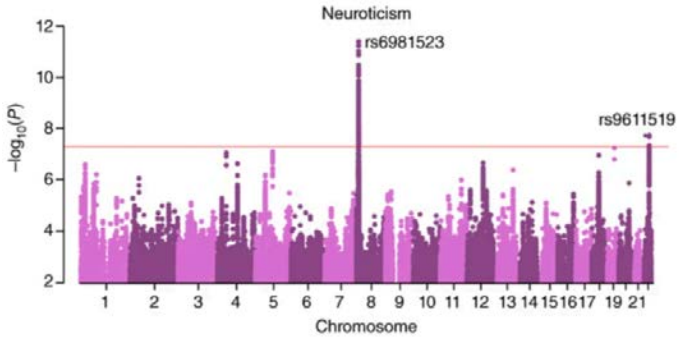


Figure 4: Manhattan plots for neuroticism. Higher peaks at a certain chromosome indicate a stronger association with that chromosome on which the genes listed previously are located on (Lo, et al, 2016)

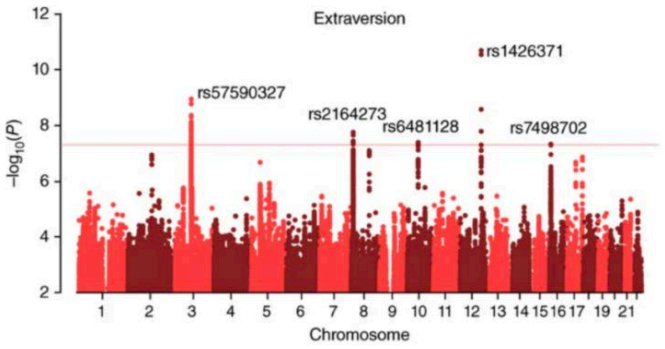


Figure 5: Manhattan plots for extraversion (Lo, et al, 2016)

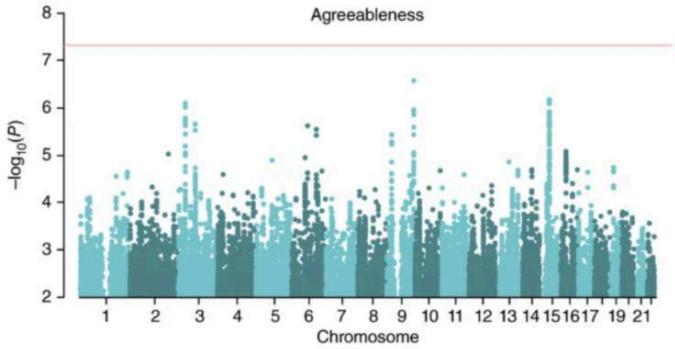


Figure 6: Manhattan plots for agreeableness (Lo, et al, 2016)

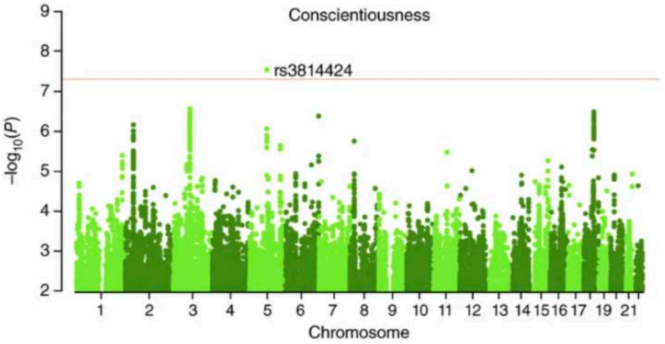


Figure 7: Manhattan plots for conscientiousness (Lo, et al, 2016)

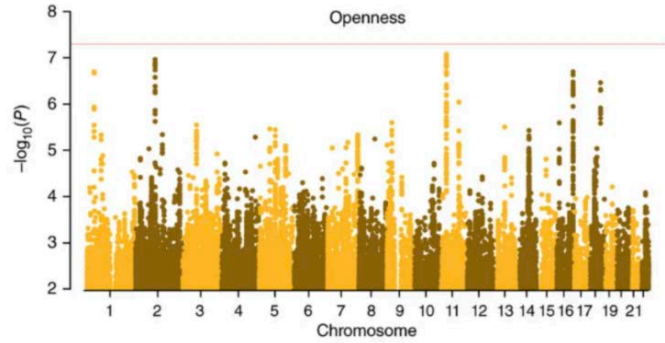


Figure 8: Manhattan plots for openness (Lo, et al, 2016)

## THE FUTURE OF GWAS AND BEHAVIORAL GENETICS

Certain behaviors can evolve into active disorders that interfere with a person's daily life. By studying GWAS and the specific genes that are associated with specific behaviors, researchers can develop therapies that target those specific genes without causing extreme, unwanted adverse effects. However, the manipulation of these genes may arguably cross the ethical line of interfering with human nature and changing who the target is as a person entirely. While the purpose of these targeted therapies is to simply help those with severe disorders and improve their lives, future parents may take advantage of these therapies to create the "perfect" child, adjusting their child's genes until they exhibit the behaviors they deem desirable. As such, the development of target therapies should be carefully regulated and not easily accessible to the public due to its overwhelming risks and ethical complications.

## CONCLUSION

Contrary to common belief, behaviors are more strongly influenced by a genetic predisposition compared to their environment effect, as explored through twin and adoption studies as well as the Genome-Wide Association Studies. However, both genes and environment will always play a role in determining the behaviors and personality traits of an individual. By recognizing and understanding how genes relate to the heritability of these behaviors, future generations can develop targeted therapies but also need to be wary of ethical concerns regarding potential misuse.

## REFERENCES

1. Campos, Adrian I. Mitchell, Brittany L. Rentería, Miguel E. (2019). "Twins Can Help Us Understand How Genes and Environment Shape Us."

2. Harris, Julie A. Lang, L Kerry. Olson, James M. Vernon, Philip A. (2001). "The Heritability of Attitudes: Twin Studies." Attitudes and Social Cognition, vol 80. Journal of Personality and Social Psychology. pp 845-46.

3. Hart, Sara et al. (2021). "Nurture might be nature: cautionary tales and proposed solutions." Science of Learning.

4. Lo, Min-Tzy. (2017). “Genome-wide analyses for personality traits identity six genomic loci and show correlations with psychiatric disorders.” Nature genetics, vol 49, 1. pp 152-156.

5. Lo, MT., Hinds, D., Tung, J. et al. (2016). “Genome-wide analyses for personality traits identify six genomic loci and show correlations with psychiatric disorders.”

6. MedicinePlus. “What are genome-wide association studies?” National Library of Medicine.

7. Myers, David G., DeWall, C. Nathan. (2018). *Psychology 12e* (12th ed.).

8. Vinkhuyzen, AAE. (2012). “Common SNPs explain some of the variation in personality dimensions of neuroticism and extraversion.” Translational psychiatry, vol 2.

# EVIDENCE FOR EVOLUTION: A BRIEF REVIEW

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**ABSTRACT**
Evolutionary theory offers a unifying framework for understanding the diversity of life on Earth. Formalized by Charles Darwin as “descent with modification” through natural selection, the concept has since been supported by extensive evidence across multiple scientific disciplines. Paleontological records reveal transitional forms and chronological patterns of increasing complexity. Comparative anatomy highlights homologous structures that point to common ancestry, while embryology demonstrates shared developmental pathways among diverse species. Molecular biology confirms genetic relationships through DNA sequence comparisons, and biogeography illustrates the role of geographic isolation in speciation. Real-time evolutionary changes, such as the emergence of antibiotic resistance, provide observable instances of natural selection. Additionally, vestigial structures and pseudogenes offer insight into evolutionary history and inherited traits that have lost their original function. Collectively, these lines of evidence converge to substantiate evolution as a foundational principle in modern biology.

## AN INTRODUCTION TO EVOLUTION

Contrary to popular belief, evolution as a concept did *not* begin with Charles Darwin. In fact, the idea arose as early as the 6th century BCE, where ancient Greek philosophers proposed similar ideas. Anaximander, for example, suggested that life originated in water and that simpler life forms gradually gave rise to more complex ones (Bowler, 2003). Similarly, Empedocles theorized that the natural world developed through a survival process, where various parts and forms of organisms randomly combined, and only those combinations that were able to survive persisted (Bowler, 2003).

It was not until Charles Darwin, however, that these early concepts were synthesized into a coherent scientific theory. Darwin defined evolution as "descent with modification," a definition that is widely accepted to this day. He theorized that evolution could be explained by a process Darwin called natural selection: changes in survival of organisms following their natural genetic variation. According to this theory, offspring have traits that differ from one another and their parents (due to natural genetic variation). These traits can make offspring more viable to survive and reproduce in an environment that has constraints on resources. As a result, these traits are more likely to be passed down than traits that make an organism *less* viable to survive (National Academy of Sciences, 1999).

The theory of evolution, as Darwin defines it, is widely accepted due to the sheer amount of supporting evidence. Supporting evidence includes, but is not limited to: fossil records, homologous structures, embryology, DNA, biogeography, vestigial traits. Additionally, real-time examples of evolution have been observed in several studies.

## THE FOSSIL RECORD

While digging canals across England, British engineer William Smith noticed that the layers of sedimentary rock followed a consistent vertical pattern across all regions. By examining the physical characteristics of these layers, he determined that the lower layers were older—a logical conclusion, since lower rock layers are deposited before those above them (Winchester, 2001). This observation led to the recognition that distinct layers contained unique groups of fossils, allowing scientists to develop a chronological understanding of fossil life based on the layers in which the fossils were found (National Academy of Sciences, 1999). Older fossils display common anatomical traits, whereas newer fossils reveal increasing complexity and speciation over time (Valentine, 2004).

For example, the fossil record reveals a gradual transition from aquatic lobe-finned fish to early amphibians. In older (and therefore deeper) layers of rock, fossils show fins with internal bones with broad, paddle-like shapes and no joints to bear weight. These fins were ideal for propulsion in water, but not suited for movement on land (Clack, 2012). In intermediate layers, transitional species left fossils that exhibit a mix of traits, including fin bones that resemble wrists and a mobile neck. These adaptations were useful for navigating shallow waters or muddy environments near shore (Daeschler et al., 2006). In younger (and therefore higher) layers of rock, fossils display fully formed limbs with multiple digits and robust skeletal structures capable of supporting the animal's body on land (Coates & Clack, 1990).

## COMPARATIVE ANATOMY

In 1555, French naturalist Pierre Belon noted that the skeletons of humans and birds were arranged similarly (Encyclopaedia Britannica). This early observation helped lay the foundation for the scientific practice of comparing the anatomical structures of different organisms, a field now known as comparative anatomy. Comparative anatomy examines the similarities and differences in the body structures of different species with the intention of understanding functional adaptations and evolutionary relationships.

A key piece of evidence for evolution found through comparative anatomy is the presence of homologous structures: anatomical features shared by different species that have a common evolutionary origin, even if they now serve different functions. A prime example of this is what Belon himself noticed: the forelimbs of humans, whales, bats, and birds all contain the same underlying bone structure, including the humerus, radius, ulna, carpals, metacarpals, and phalanges. These structures have been

slightly modified over time to support different functions such as grasping, swimming, flying, and perching, but overall share the same shape (National Academy of Sciences, 1999). This shared structural pattern strongly suggests that these species evolved from a common ancestor with a similar limb design.

**EMBRYOLOGY**

Embryology is the branch of biology that examines the development of organisms from fertilization to birth. There is a remarkable similarity between early developmental stages of distinct species. In initial stages, for example, embryos of fish, birds, and mammals share anatomical features such as gill slits, tails, and segmented body structures. These features are present in embryos, regardless of whether or not they are later lost as development progresses (Lyson, Bever, Bhullar, Joyce, & Gauthier, 2010).

These developmental patterns support the theory that different species evolved from an ancestral species that possessed these characteristics. Evolution, by nature, preserves deeply rooted genetic and developmental pathways. While later developmental stages can undergo significant modifications through natural selection to produce species-specific adaptations, the fundamental embryonic blueprint remains largely unchanged due to its critical role in establishing basic body plans (Lyson, Bever, Bhullar, Joyce, & Gauthier, 2010). Over time, natural selection and genetic mutations have reshaped these embryonic structures to serve specialized functions in different lineages. For example, while gill slits develop into functional respiratory organs in fish, they contribute to structures like the Eustachian tubes in mammals or are entirely repurposed in other species (Encyclopaedia Britanica). Interestingly, embryology helps explain rare instances where ancestral traits reappear (atavisms), such as hind limb buds in whales or extra digits in horses. This is evidence that the genetic instructions for these features still exist, even if they are suppressed during development (Lyson, Bever, Bhullar, Joyce, & Gauthier, 2010).

**MOLECULAR BIOLOGY**

DNA (or deoxyribonucleic acid) is the molecule that stores genetic information. Despite the wide diversity of life on Earth, DNA is remarkably similar across even vastly different species, implying that they all share a common ancestry (National Academy of Sciences, 1999). By comparing DNA sequences, biologists are able to determine not only how closely related two organisms are, but also estimate when they diverged from a common ancestor. For example, studies have shown that humans and chimpanzees share approximately 98.7% to 99% of their DNA sequences (Britten, 2002; The Chimpanzee Sequencing and Analysis Consortium, 2005). This high level of similarity points to a recent common ancestor in evolutionary terms. Based on molecular clock analyses—which estimate the rate at which genetic mutations accumulate—scientists estimate that humans and chimpanzees diverged from a common ancestor between 5 and 7 million years ago (Langergraber et al., 2012; Patterson et al., 2006).

**BIOGEOGRAPHY**

The geographic distribution of species often provides compelling evidence for their evolutionary history. This field of study, known as biogeography, examines how and why organisms are distributed across different regions of the Earth (Lomolino et al., 2010). Species that evolve in geographic isolation frequently develop unique adaptations in response to their specific environments. A classic example is the Galápagos finches, which Charles Darwin observed during his voyage on the *HMS Beagle* (Darwin, 1859/2009). These birds, though descended from a common ancestor, evolved different beak shapes and sizes to exploit distinct food sources on the islands (Grant & Grant, 2002). The process through which a single ancestral species diversifies into multiple species adapted to different ecological niches is known as adaptive radiation (Schluter, 2000), and it plays a central role in the generation of biodiversity.

**OBSERVED EVOLUTION**

While much of the evidence for evolution comes from ancient history, scientists have also observed evolution occurring in real time. One of the most famous (and concerning) examples is the development of antibiotic resistance in bacteria. Because bacteria reproduce rapidly, spontaneous mutations can quickly lead to new traits, including resistance to antibiotics. When

antibiotics are introduced, they kill susceptible bacteria, but those with resistant traits survive and reproduce. Over time, these resistant strains become more prevalent, demonstrating natural selection in action (Baym et al., 2016). A demonstration of this process was created by researchers at Harvard Medical School, who constructed a giant petri dish (called the MEGA-plate) to observe how *Escherichia coli* bacteria evolved resistance to antibiotics over time and space (Baym et al., 2016; AAAS, 2016). As the bacteria migrated into zones with higher antibiotic concentrations, they developed successive mutations that enabled them to survive and expand into previously uninhabitable areas.

**VESTIGIAL STRUCTURES**

Vestigial structures are anatomical features that have lost their original function over time but remain present in an organism. These structures are remnants of an evolutionary past and serve as evidence for common ancestry (Hall, 2012). For example, whales and some snakes retain small, non-functional pelvic bones, suggesting that their ancestors had limbs and lived on land (Thewissen et al., 2006; Wiens, Brandley, & Reeder, 2006).

In humans, the appendix, wisdom teeth, and tailbone (coccyx) are considered vestigial, hinting at a time when these features served important functions in our evolutionary ancestors (Smith & Morton, 2001). Additionally, the presence of pseudogenes (non-functional remnants of once-useful genes) further supports the idea that species have evolved from common ancestors through a process of descent with modification (Lynch, 2007; Max, 2003).

**CONCLUSION**

The evidence supporting evolution is extensive and comes from a wide range of scientific disciplines, including paleontology, genetics, comparative anatomy, and ecology. Together, these fields provide a consistent and well-supported explanation for how life has changed over time. They show the shared characteristics among diverse species, revealing patterns that point to common origins and a long history of gradual change shaped by natural processes. Ongoing discoveries add to the growing pile of evidence.

**REFERENCES**


1. "Comparative Anatomy." *Encyclopaedia Britannica*, Encyclopaedia Britannica, Inc.,

2. Baym, Michael, et al. "Spatiotemporal Microbial Evolution on Antibiotic Landscapes." *Science*, vol. 353, no. 6304, 2016, pp. 1147–1151.

3. Bowler, Peter J. *Evolution: The History of an Idea*. 3rd ed., University of California Press.

4. Clack, Jennifer A. *Gaining Ground: The Origin and Evolution of Tetrapods*. 2nd ed., Indiana University Press, 2012.

5. Coates, Michael I., and Jennifer A. Clack. "Polydactyly in the Earliest Known Tetrapod Limbs." *Nature*, vol. 347, no. 6288, 1990, pp. 66–69.

6. Daeschler, Edward B., Neil H. Shubin, and Farish A. Jenkins. "A Devonian Tetrapod-like Fish and the Evolution of the Tetrapod Body Plan." *Nature*, vol. 440, no. 7085, 2006, pp. 757–763.

7. Darwin, Charles. *On the Origin of Species*. 1859. Harvard University Press, 2009.

8. Grant, Peter R., and B. Rosemary Grant. "Adaptive Radiation of Darwin's Finches." *American Scientist*, vol. 90, no. 2, 2002

9. Hall, Brian K. *Evolution: Principles and Processes*. Jones & Bartlett Learning, 2012.

10. Langergraber, Kevin E., et al. "Generation Times in Wild Chimpanzees and Gorillas Suggest Earlier Divergence Times in Great Ape and Human Evolution." *Proceedings of the National Academy of Sciences*, vol. 109, no. 39, 2012, pp. 15716–15721.

11. Lomolino, Mark V., et al. *Biogeography*. 4th ed., Sinauer Associates, 2010.

12. Lynch, Michael. *The Origins of Genome Architecture*. Sinauer Associates, 2007.

13. Max, Edward E. "Plagiarized Errors and Molecular Genetics: Another Argument in the Evolution-Creation Controversy."
*Why Intelligent Design Fails*, edited by Matt Young and Taner Edis, Rutgers University Press, 2003, pp. 44–82.

14. National Academy of Sciences. *Science and Creationism: A View from the National Academy of Sciences*. 2nd ed., National Academies Press, 1999.

15. Patterson, Nick, et al. "Genetic Evidence for Complex Speciation of Humans and Chimpanzees." *Nature*, vol. 441, no. 7097, 2006, pp. 1103–1108.

16. Russo, Claudia A. M., et al. "Science and Evolution." *Genetics and Molecular Biology*, vol. 42, no. 1 suppl 1, 2019, pp. 1–6.

17. Smith, Holly F., and Deborah G. Morton. "The Human Tail: Functional Vestigiality or Evolutionary Relict?" *Clinical Anatomy*, vol. 14, no. 1, 2001, pp. 51–54.

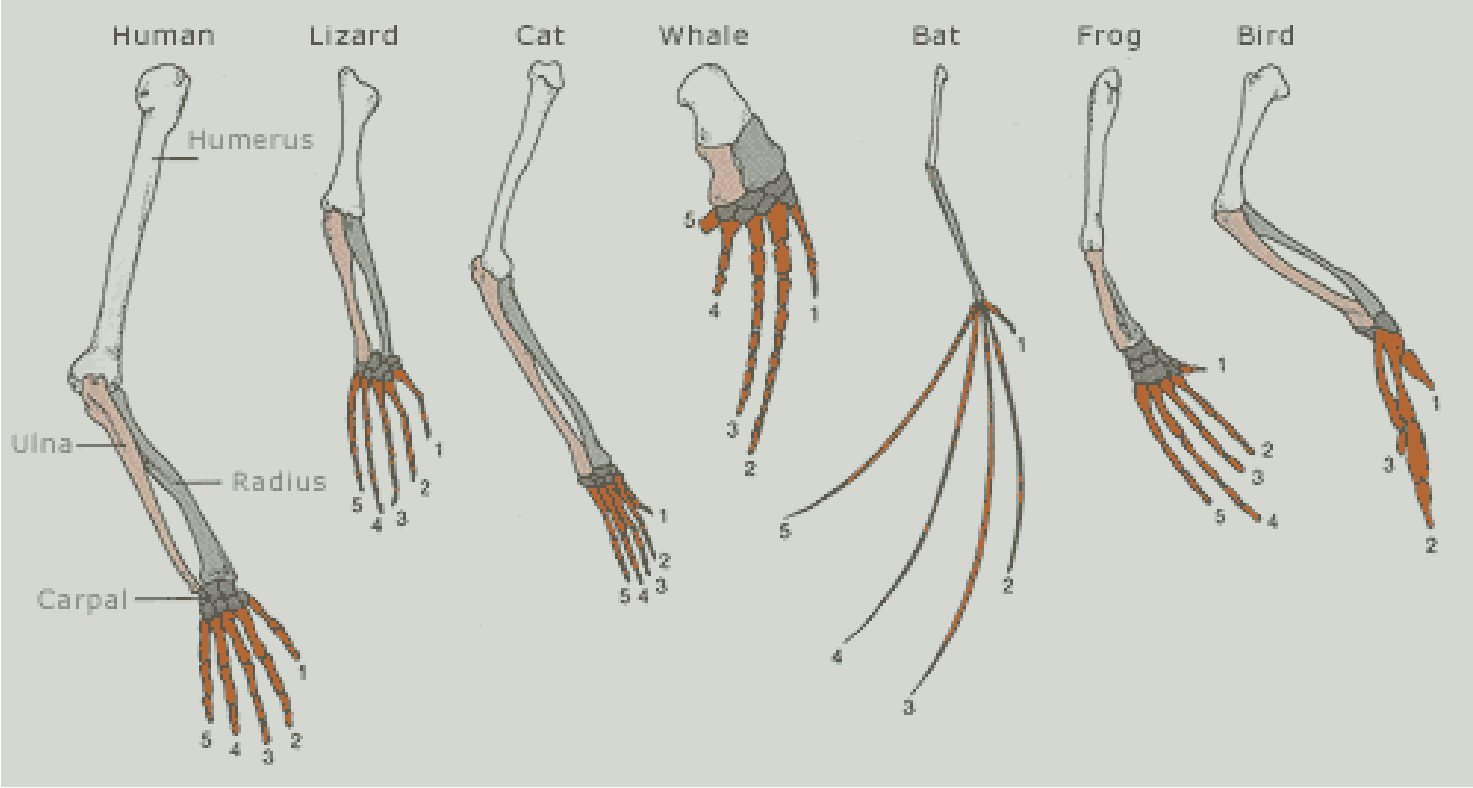
18. The Chimpanzee Sequencing and Analysis Consortium. "Initial Sequence of the Chimpanzee Genome and Comparison with the Human Genome." *Nature*, vol. 437, no. 7055, 2005, pp. 69–87.

19. Thewissen, J. G. M., et al. "Whales Originated from Aquatic Artiodactyls in the Eocene Epoch of India." *Nature*, vol. 450, no. 7173, 2007, pp. 1190–1194.

20. Valentine, James W. *On the Origin of Phyla*. University of Chicago Press, 2004.

21. Wiens, John J., et al. "Why Does a Trait Evolve Multiple Times Within a Clade? Repeated Evolution of Snake-like Body Form in Squamate Reptiles." *Evolution*, vol. 60, no. 1, 2006, pp. 123–141.

22. Winchester, Simon. *The Map That Changed the World: William Smith and the Birth of Modern Geology*. HarperCollins, 2001.




Comparative anatomy of forelimbs in various vertebrates, including a human, lizard, cat, whale, bat, frog, and bird. Despite differences in function—such as grasping, walking, swimming, or flying—all forelimbs share a common structural pattern composed of the humerus, radius, ulna, carpals, and phalanges. This illustrates the concept of homologous structures, providing strong evidence for common ancestry and divergent evolution in vertebrates.

*Image citation: OpenStax College. (n.d.). How do we know evolution has occurred? Comparative anatomy. In Evolution and Biology. University of Minnesota Libraries Publishing.*

# FROM STINGERS TO BUILDERS: DISCOVERING THE FASCINATING TIES BETWEEN JELLYFISH AND CORAL

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**ABSTRACT**
Although jellyfish and corals appear vastly different, they are surprisingly close relatives, both belonging to the ancient phylum *Cnidaria*. Characterized by radial symmetry and stinging cells called nematocysts, cnidarians exhibit two primary life forms: the free-swimming medusa (e.g., jellyfish) and the sessile polyp (e.g., corals). Despite their shared lineage, jellyfish and corals play vastly different roles in marine ecosystems. Jellyfish are efficient carnivorous predators that help regulate marine populations, while corals act as reef-building organisms essential for biodiversity. Coral survival depends on a delicate symbiosis with photosynthetic algae, but this relationship is threatened by climate change, resulting in coral bleaching and reef decline. Meanwhile, jellyfish populations are surging due to warming oceans and reduced predation, leading to ecological disruptions. Understanding the biology and environmental challenges of these cnidarians highlights the urgent need for conservation efforts to maintain marine ecosystem balance.

## INTRODUCTION

At first glance, jellyfish and corals might seem like two completely different creatures. The only similarity they share appears to be that they both inhabit the ocean—and even this is far-fetched, as one floats freely, and one remains anchored to the seafloor. However, as surprising it may seem, they are much closer relatives than one might think! Both belong to the phylum *Cnidaria*, which is part of an ancient group of animals that show radial symmetry (Digital Atlas of Ancient Life).

Figure 1: A jellyfish (Chetan-Welsh)

Figure 2: A coral outcrop on the Great Barrier Reef, Australia (Natural Science Foundation, 2024)

## RADIAL SYMMETRY

Radial symmetry is akin to a pizza—just like slices of pizza radiate evenly from the center, the body parts of cnidarians are arranged around a central point, allowing them to interact with the environment in all directions. Another defining trait of Cnidarians are their unique stinging structures, known as nematocysts, which reside in specialized tentacle cells and enable these animals to capture small prey (Özbek et al., 2012).

## LIFE STAGES AND BEHAVIORS OF JELLYFISH AND CORALS

Although jellyfish and coral share a common evolutionary ancestry, they exhibit different physical characteristics. This difference stems from two separate primary life stages of Cnidarians: the polyp and the medusa. The polyp exists as a fixed, sac-like shape, while the medusa is a free-swimming bell-shaped form (BYJU'S). Corals spend their lives anchored as polyps, building massive reef structures, while jellyfish primarily exist in the medusa form, drifting through open water. Although they appear fragile, jellyfish function as efficient predators by using their stinging cells to capture prey. The stinging cells, embedded in their tentacles, number in the thousands and activate upon contact to deliver toxins which paralyze fish and plankton (ScienceDaily, 2017). The carnivorous eating habits of jellyfish play a crucial role in maintaining marine ecosystem stability because they help control population numbers of other marine life.

In contrast to jellyfish, corals are the architects of the ocean. These small, sessile organisms create massive coral reefs by secreting calcium carbonate exoskeletons, forming intricate structures that provide habitats for a wide variety of marine species (Woods Hole Oceanographic Institution, 2018). Corals are made up of tiny polyps—each one a small, soft-bodied animal that resembles a tiny flower with tentacles around its mouth. These polyps often live in colonies, and together they form the colorful and complex coral reefs we see in tropical oceans.

A key factor behind their vibrant colors is tiny algae called zooxanthellae. These algae live inside the coral's tissue, where they photosynthesize and provide the coral with nutrients. In return, the coral provides the zooxanthellae with a safe environment and carbon dioxide (NOAA). This symbiotic relationship is what allows coral reefs to thrive in nutrient-poor waters and gives them

their stunning hues.

**ECOSYSTEM CONSIDERATIONS**

Nowadays, corals face severe threats to their survival due to climate change and ocean pollution. One of the most devastating effects is coral bleaching. This phenomenon occurs when corals expel their symbiotic algae due to environmental stress like rising ocean temperatures or ocean acidification (Great Barrier Reef Foundation). Without their algae partners, corals lose their color and, more importantly, their primary source of food, leaving them vulnerable to disease and death.

While corals struggle to survive, jellyfish populations are booming. Warming ocean temperatures, increased pollution, and intensive fishing (which depletes some of jellyfish's natural predators) cause jellyfish populations to surge.

The booming population of jellyfish has caused vast numbers of bottleneck jellyfish to be washed ashore by strong winds, where they sting thousands of people. Large jellyfish blooms can also block the cooling systems of nuclear power plants, clog fishing nets, and outcompete fish for food (JSTOR Daily, 2016).

To restore ecological balance, humans have put effort into mitigating climate change, either by reducing greenhouse gas emissions from burning of fossil fuels or enhancing natural carbon sinks (like oceans and forests) that accumulate and store these gases. With continued effort, humans can restore the health of the oceans and the diversity of the vibrant life in them.

**REFERENCES**


1. BYJU'S. "Difference Between Polyp and Medusa." *BYJU'S.*

2. Castro, Peter, and Michael E. Huber. *Marine Biology*. 10th ed., McGraw-Hill Education, 2016, pp. 121–122.

3. Digital Atlas of Ancient Life. "Cnidaria." *Digital Atlas of Ancient Life.*

4. Great Barrier Reef Foundation. "Understanding Coral Bleaching." *Great Barrier Reef Foundation.*

5. JSTOR Daily. "The Global Jellyfish Crisis in Perspective." *JSTOR Daily*, 2016.

6. National Science Foundation. "Water Temperature on Australia's Great Barrier Reef Hottest in 400 Years." *NSF.gov*, 17 Mar. 2020.

7. Natural History Museum. "What Is a Jellyfish?" *NHM.ac.uk.*

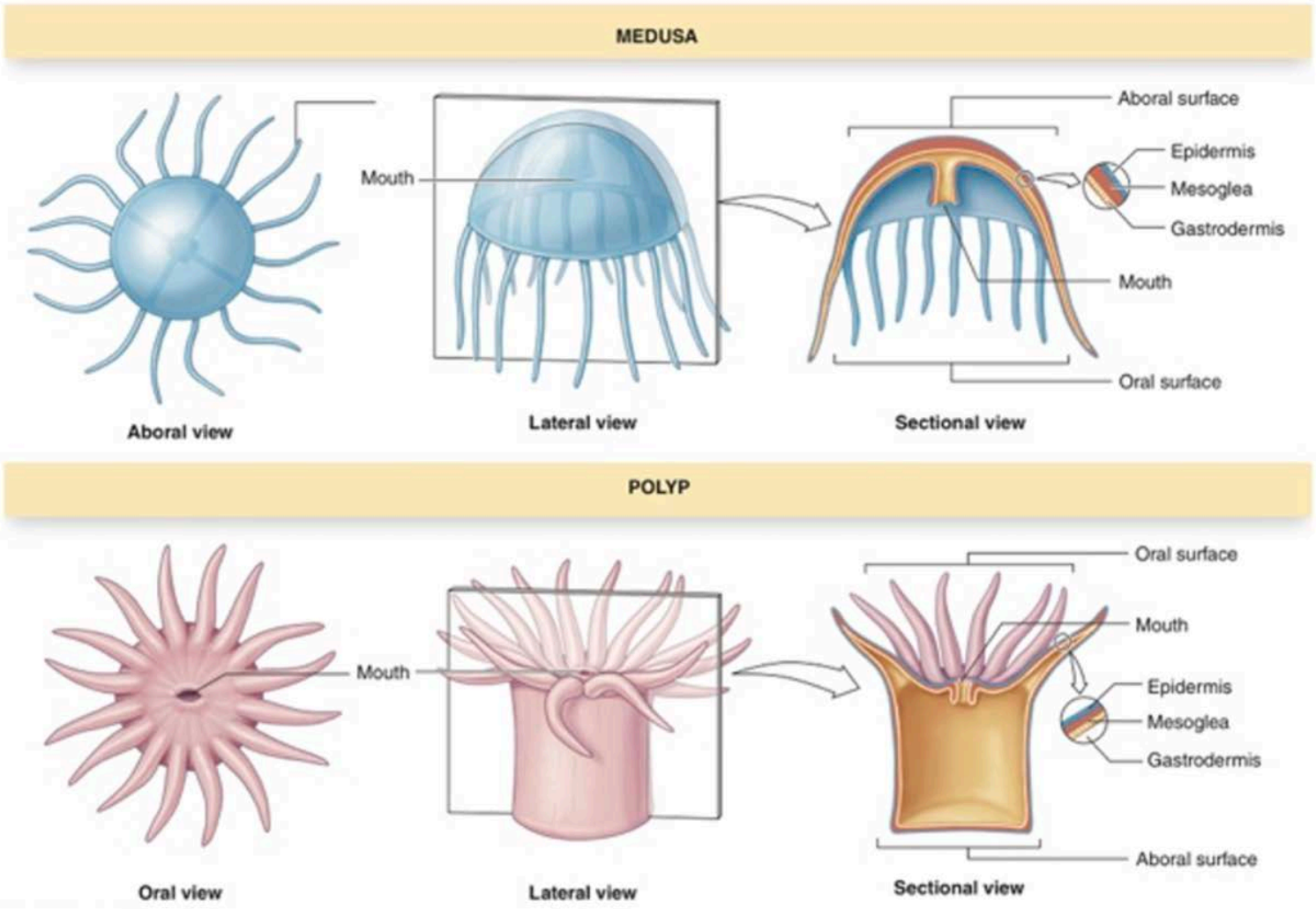
8. NOAA. "What Is Zooxanthellae?" *National Ocean Service.*

9. Nunez, Kali. "Are You Ready to Keep Jellyfish?" *AlgaeBarn*, 23 Feb. 2021.

10. Özbek, Suat, P. G. Balasubramanian, and Thomas W. Holstein. "The Nematocyst: A Molecular Map of the Cnidarian Stinging Organelle." *The International Journal of Developmental Biology*, vol. 56, no. 6–8, 2012, pp. 577–582.

11. ScienceDaily. "Jellyfish: Stinging Cells Pack a Powerful Pressure." *ScienceDaily*, 20 Nov. 2017.

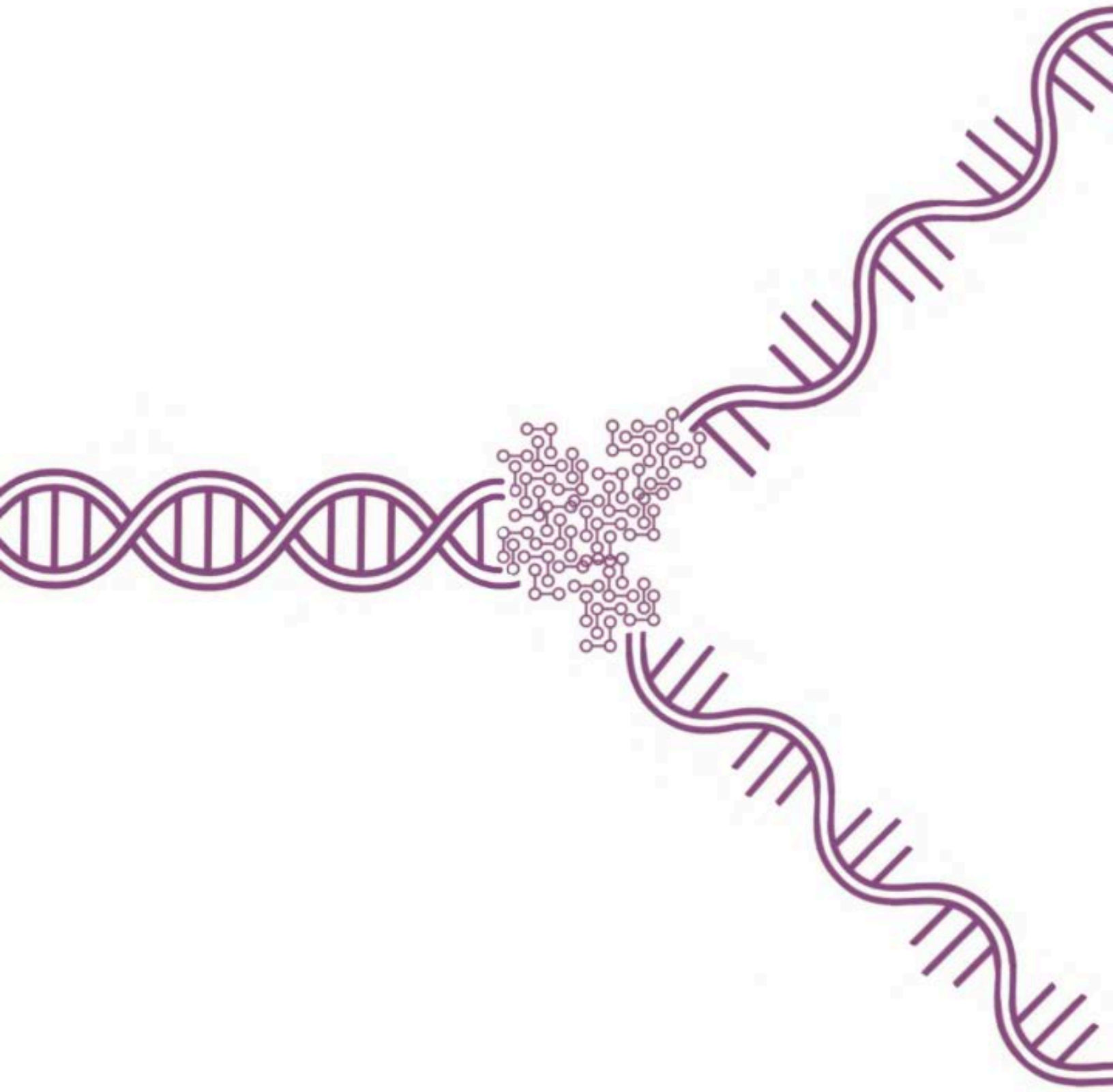
12. Woods Hole Oceanographic Institution. "How Do Corals Build Their Skeletons?" *WHOI.edu.*




A depiction of the difference between the medusa and the polyp. The flower-like appearance of many cnidarians is a consequence of their radial symmetry. In both the medusa and the polyp, tentacles are arranged and repeated around a central axis that runs through the mouth.

*Image credits: Castro, Peter, and Michael E. Huber. Marine Biology. 10th ed., McGraw-Hill Education, 2016*

# RESEARCH PAPERS



# SEMAGLUTIDE: A REVOLUTIONARY DIABETES DRUG, OR A FAD CELEBRITY ENDORSEMENT?

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**ABSTRACT**
This comprehensive review explores the social and health effects of semaglutide, a GLP-1 receptor agonist, and evaluates its efficacy as a treatment for Type 2 diabetes. This paper delves into its mechanism of action, detailing its similarities to the glucagon-like peptide-1 (GLP-1) hormone to regulate insulin secretion, slow gastric emptying, and reduce appetite. Additionally, it examines the drug's impact on various physiological systems, including its cardiovascular benefits and potential side effects such as gastrointestinal distress, pancreatitis, and potential long-term risks. Furthermore, this review compares semaglutide to other widely used diabetes medications, such as insulin and metformin, assessing its advantages and limitations in glycemic control and other factors, such as weight loss and frequency of cardiovascular events by analyzing clinical data. Beyond its medical applications, this review investigates semaglutide's rising status as a social drug, particularly in the context of weight loss. The influence of celebrity endorsements and media representation has contributed to a surge in demand for semaglutide as an off-label weight loss solution, affecting public perception and access to the medication. This review aims to explore the societal effects of these endorsements, particularly their impact on body image, healthcare accessibility through the lens of social media and semaglutide's availability for diabetes patients. Additionally, this review evaluates the implications of semaglutide's popularity on healthcare equity, particularly for low-income and uninsured populations. With high costs and limited insurance coverage, semaglutide's accessibility remains a critical issue, potentially exacerbating health disparities and limiting treatment options for those who need it most. The paper will also consider future developments, including emerging alternatives and innovations in diabetes treatment, assessing how they might address these challenges. By synthesising medical research, social commentary, and healthcare policy discussions, this review provides a comprehensive analysis of semaglutide's role in modern medicine and society. It aims to offer insight into the broader implications of its rise as both a diabetes treatment and a social phenomenon, ultimately assessing its potential benefits and ethical concerns in public health.

## INTRODUCTION

With the ever changing nature of the healthcare industry, breakthroughs in any field are sure to catch the public and scientific communities' attention. One such breakthrough has been semaglutide, an injectable medication developed to treat type 2 diabetes. While effective at lowering blood sugar levels and promoting weight loss, its fame is thanks to a combination of celebrity influence and social media. Having made its way onto several platforms, its popularity has soared over the past few months. Semaglutide is also known more widely as its brand name, Ozempic (a drug that's FDA approved to treat type 2 diabetes) and Wegovy (a drug that's FDA approved for weight loss); both of which will be used to reference the drug. The treatments have the same active ingredients, except Wegovy has a higher maximum dose of 2.4 mg compared to 2.0 mg of Ozempic (Wegovy®, n.d.). The objective of this project is to unfold the social and health benefits, drawbacks, and grey areas with regards to the recent surge in demand for both medications.

This review aims to separate the fame from science and shed light on the true impact that the drug has had on individuals with type 2 diabetes and the healthcare system. In addition, this review will deeply scrutinize its value as a genuine innovation to improve quality of life in patients with diabetes in comparison to its widespread rumours and misconceptions as a 'miracle' drug amongst the general public.

## THE MECHANISMS OF SEMAGLUTIDE

Semaglutide is prescribed as an injector pen of a specific dosage, which patients inject into their lower abdomen, and belongs to a family of drugs known as glucagon-like-peptide (GLP-1) receptor agonists. These drugs mimic a hormone that stimulates the pancreas to release insulin, while suppressing the release of another hormone called glucagon, which in turn helps reduce hunger (Els, 2024). Its structure consists of 31 amino acids, linearly joined through peptide bonds, having a 94% similarity to human GLP-1 (Kalra & Sahay, 2020). It does, however, contain amino acid substitutions at amino acid position 8. Substituting the amino acid alanine to a-methyl amine prevents dipeptidyl peptidase-4 (DPP-4) degeneration, which enhances the uptake of glucose by cells. It also includes a substitution at position 34, replacing lysine with arginine. This helps it bind to albumin; a prominent protein found in blood plasma.

Semaglutide works in 3 main ways: by increasing insulin production, inhibiting the release of glucagon, and slowing gastric emptying—which increases the feeling of satiety (Mahapatra, Karuppasamy, & Sahoo, 2022). In addition, its main mechanisms involve the inhibition of glucagon release and suppression of hepatic gluconeogenesis, a process that forms glucose from various other non-carbohydrate, organic compounds. Overall, this augments insulin production, increasing glucose uptake into cells, and thus lowering blood sugar levels in type 2 diabetics.

Additionally, the interaction of GLP-1 and its receptor, GLP-1R, happens through a mechanism that ultimately increases insulin production. First, GLP-1 binds to GLP-1R, which then stimulates the release of the enzyme adenylyl cyclase, stimulating the production of a messenger (adenosine monophosphate, or cAMP) from ATP. This triggers the release of protein kinase A (PKA), which can close the K+ ion channel, causing the voltage-dependent Ca2+ channel to open (Klec, Ziomek, Pichler, Malli, & Graier, 2019). Further pathways additionally increase the Ca2+ concentration inside the cell. This ion plays a significant role in the Krebs cycle on dehydrogenases, enzymes that stimulate ATP production, which in turn has a positive correlation with the glucose intake of cells (Traaseth, Elfering, Solien, Haynes, & Giulivi, 2004).

Another way that semaglutide works is by inhibiting glucagon. Within type 2 diabetes, elevated glucagon levels result in hyperglycaemia. The drug works with insulin homeostatically to regulate blood glucose levels and is counterregulatory to insulin and catabolic in nature, meaning it breaks down molecules. Glucagon is released by alpha cells in the pancreas, but in type 2 diabetes, these cells are dysfunctional, causing hyperglycaemia in fasted states and after food intake (Rix et al., 2019). The mechanism behind this works similarly to the release of insulin from beta cells, which are cells in the pancreas that make insulin. First, glucose is uptaken by the glucose

transporter 1 in the cell membrane, which goes through glycolysis to ultimately produce ATP. However, since this is reflective of blood glucose levels, lower ATP production closes K+ ion channels, which are ATP-dependent. As a result, this causes depolarisation of the cell, thus opening the Ca2+ channels, which are the main trigger for the release of glucagon into the extracellular space (Zhao et al., 2021).

These mechanisms can thus influence organs. In the liver, fat content, glucose production, and plasma enzymes decrease. As a result, this keeps blood sugar levels down. In the pancreas, insulin secretion increases, therefore increasing the efficiency at which cells take up glucose, therefore decreasing blood glucose levels (Pang, Feng, Ling, & Jin, 2022). Due to slower gastric emptying, the feeling of satiety increases, which can help in the weight loss to decrease complications from type 2 diabetes patients. As a result, less ghrelin (the 'hunger' hormone) is released from the stomach, instead promoting the release of leptin (the 'fullness' hormone) and subsequently decreased appetite.

## EVALUATING SEMAGLUTIDE AS A TREATMENT FOR DIABETES

### EFFECT ON HbA1C LEVELS

HbA1c levels refer to glycated haemoglobin levels and are the primary measurement to assess blood sugar levels. A reduction in HbA1c levels indicates a better control of blood sugar and is thus important to measure efficacy of semaglutide as a treatment for diabetes.

According to a meta-analysis by Y. Al Hindi and A. Avery, a 14.0 mg dose of oral semaglutide significantly reduced HbA1c levels, the mean difference being a reduction of 1.30%. In another study from the PIONEER phase 3 program, a large-scale study that assessed the efficacy and safety of oral semaglutide, patients with higher baseline HbA1c levels had a higher decrease in mean HbA1c levels (Aroda et al., 2022). The mean percentage difference was 1.7%- 2.6% for those with levels above 9.0%. In some cases, some ethnicities such as Asians had a larger percentage decrease with a 14 mg dose of oral semaglutide, from around -1.5% to 1.8% for the baseline of more than 7% (Buse et al., 2020). In another phase of the PIONEER program, it was found that with flexible dosage, the mean difference in glycated haemoglobin levels was 0.1%, and 52% of patients achieved an HbA1c level below 7% with oral semaglutide. These statistics provide significant evidence for improvement in blood sugar levels, which is important for patients suffering from diabetes as it reduces the risk of diabetes related complications like diabetic retinopathy (vision loss), kidney disease and cardiovascular diseases.

However, do subcutaneous forms of semaglutide have a higher efficacy? In one study, patients who were considered 'pre-diabetic', and had a HbA1c level of 8.0% to 8.1%, with a subcutaneous dose of 14 mg of semaglutide were found to have a reduction of 1.6% at 30 weeks of medication (Meier, 2021). This was superior to the placebo. For people with diabetes, who had a mean initial HbA1c level of 62 mmol/mol, the mean decrease in HbA1c was -12.6 mmol/mol (the naïve group). Those who were 'experienced' with semaglutide had a mean reduction by 5.6 mmol/mol, with each group reaching levels below 54 mmol/mol within 2 years (Vilsbøll, Lindahl, Nielsen, & Tikkanen, 2023).

Another study done by Novo Nordisk, the company that produced Ozempic, found that there was a 77% decrease in Fasting plasma glucose (FPG) levels (44 from 191), along with 62% of people reaching HbA1c levels under less than 7% within 56 weeks (Novo Nordisk, 2020). However, since the study was funded and carried out with the associated institution, it is important to note that it may have been subject to funding bias.

In conclusion, these studies, when compiled together, show an overall efficacy in reducing Hb1Ac levels. Additionally, most statistical t-tests show mean reductions at the 5% significance level to be less than or equal to the critical value, showing a significant improvement in HbA1c and FPG levels from baseline.

### WEIGHT LOSS EFFECTS

In a landmark double-blind study, participants on semaglutide dose of 2.4 mg showed a mean weight loss of 14.9% in 68 weeks from their baseline, compared to the control group of 2.9%, which contained 655 individuals over the

age of 18 without diabetes (Wilding et al., 2021).

An additional study showed that the mean weight loss of 5.9% after 3 months, whereas after 6 months 10.9% in patients without diabetes (Ghusn et al., 2022). However, patients with type 2 diabetes lost less weight than those without type 2 diabetes, with a mean of 3.9% of patients with diabetes, and 6% for patients without diabetes.

Further evidence is shown through a randomised clinical trial that took place as part of the STEP 3 trials. This trial was a 68-week phase 3a trial that aimed to examine weight loss effects of semaglutide in overweight or obese patients (Wadden et al., 2021). Overall, it showed a decrease in body weight of 16% for the semaglutide group, and 5.7% for the placebo group, with higher proportions of participants on semaglutide showing at least a 15% decrease in body weight. However, gastrointestinal complaints were more frequent within the semaglutide test group. 82% of participants submitted complaints, whereas this number was 19.6% lower in the placebo group. It is also important to note that participants had been placed on lower energy diets, along with intense behavioural therapy of around 30 sessions over the 68-week period for the trial.

However, it is important to note that most of the studies analysed above have used BMI as their main indicator of weight change. Even though it is an extremely common and useful quantitative value to use, it still may not be a fully valid indicator of improved health outcomes due to its inaccuracies in measuring actual fat loss, as it only takes mass into account. Therefore, valid conclusions about reduced relative risk of diabetes may not be able to be drawn. However, the HbA1c levels, along with accurate indicators such as waist-to-hip ratio throughout the mentioned studies may show that semaglutide is a valid drug to be used in supervised weight loss.

**CARDIAC IMPLICATIONS**

One study showed a rate of non-fatal stroke in 1.6% of patients who received semaglutide, compared to 2.7% of patients who received a placebo (Marso et al., 2016). Additionally, the rates of non-fatal heart attacks in the semaglutide versus placebo group were 2.9% and 3.9% respectively. However, 83% of the patients were said to have already established cardiovascular disease, chronic kidney disease or both. Therefore, it is important to note that the number of cardiac events that occurred in this trial may have been higher than that of a trial that selected patients who only had type 2 diabetes and no other cardiac conditions. Additionally, this study was funded by Novo Nordisk, the company that produces a commercial injection of semaglutide, meaning that the study may have been subject to funding bias.

In the SUSTAIN trials, the probability of a cardiac outcome was calculated using the Ghosh-Lin estimator, which is a statistical method used to analyze recurrent events. In this case, the expected number of hospitalisations due to heart failure over a time (Rogers, Yaroshinsky, Pocock, Stokar, & Pogoda, 2016). It was found that the risk of a cardiac event was significantly lower within the semaglutide group than within the placebo, with a hazard ratio of 0.74. This means that there is a 26% lower chance of the semaglutide group experiencing a cardiac event. The graph from the study is attached on the left. It shows the relationship between time and the probability of another event, with a clear correlation of lower probability of a cardiac event with a placebo compared to semaglutide (Kolkailah, Lingvay, Dobrecky-Mery, et al., 2023).

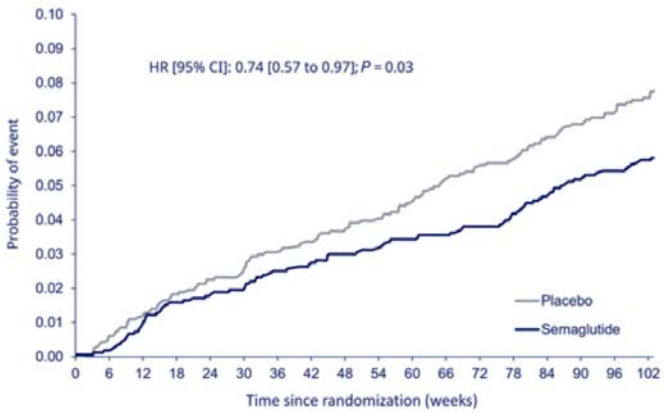


Figure 1: A Kaplan-Meier curve showing the cumulative probability of a cardiovascular event over 104 weeks in patients receiving semaglutide versus placebo (Marso et al., 2016).

Additionally, semaglutide's mechanisms involve improving lipid profiles, as well as reducing

blood pressure. For instance, semaglutide increases levels of high-density lipoprotein (HDL) while decreasing levels of low-density lipoprotein (LDL). This suggests that semaglutide may be protective against cardiac events and atherosclerosis.

Overall, throughout all the implications of semaglutide, it can be seen as an effective treatment for type 2 diabetes, with several clinical trials showing promise in not only lowering blood sugar levels but also decreasing risks of cardiac events and obesity. These factors may help patients improve quality of life and potentially achieve remission.

**EFFICACY OF SEMAGLUTIDE COMPARED TO OTHER MEDICATIONS**

In the following section, several common diabetes medications will be addressed, many of which belong to a different class of drugs as compared to semaglutide. By assessing medications in different classes, it will allow for different mechanisms to be compared and assess their advantages and disadvantages for patients with diabetes. Their mechanisms, efficacy, and benefits and limitations compared to semaglutide will be discussed.

**METFORMIN**

Metformin is usually the first line of treatment for type 2 diabetes, and belongs to a class of drugs called biguanides, which work by reducing liver glucose production and decreasing intestinal uptake of glucose without affecting the amount of insulin released from the pancreas (DrugBank, n.d.). Its mechanism is different to that of semaglutide, as it does not increase insulin secretion to control blood sugar levels. Additionally, metformin has shown evidence to be one of the only medications suitable for children from the ages of 10-16 for the treatment of type 2 diabetes and prevent pre-diabetes in children from progressing into type 2 diabetes. This is because of its safety and lower risk for diabetic ketoacidosis, which is known to cause several deaths in emerging and developing countries (Poovazhagi, 2014; Soliman, De Sanctis, Alaaraj, & Hamed, 2020). Metformin has also been shown to improve insulin sensitivity and induce ovulation in people with polycystic ovarian syndrome (PCOS): one study showed an ovulation rate of 100% with metformin as compared to 37% with a placebo (Practice Committee of the American Society for Reproductive Medicine, 2017). With rates of obesity and other risk factors for type 2 diabetes increasing with time, this may make metformin a valuable drug for treatment of insulin sensitivity in women and children. However, semaglutide was only recently approved by the US food and drug administration for use in treatment of obesity in teenagers but has not been approved for paediatric type 2 diabetes, instead being used only in adults (Novo Nordisk USA, 2022). However, weight management with semaglutide may decrease risk of teens developing type 2 diabetes later as adults.

As mentioned before, metformin has also shown incidences of lactic acidosis. Although the prevalence of lactic acidosis with metformin is low, there is still some concern with its complications. This may be due to its mechanism blocking an enzyme called pyruvate carboxylase, which acts during the first step of gluconeogenesis, a metabolic process by which glucose is produced in the liver (Blough, Moreland, & Mora, 2015). This may lead to a build-up of lactic acid in the bloodstream because of inhibited mitochondrial respiration in the liver. However, the instances of this have been rare, and can be prevented through careful prescription (DeFronzo, Fleming, Chen, & Bicsak, 2016). For instance, if a patient has liver or kidney problems, they may be prescribed a different medication instead.

Overall, because of its safety for children, metformin may be a superior drug that is applicable to a wider population than just adults with diabetes. However, due to lactic acidosis and liver/kidney problems, this drug may not be as suitable for certain conditions.

**LIRAGLUTIDE**

Liraglutide belongs to the same class of drugs as semaglutide, acting as a GLP-1 receptor agonist and having similar effects on the body to control glucose levels like semaglutide.

To examine its efficacy against semaglutide, one study showed a decrease in weight of 15.8% for the semaglutide group compared to a decrease of 6.4% in the liraglutide group. However, the study was done on adults without type 2 diabetes (Rubino et al., 2022). Looking at

its efficacy for reducing blood sugar levels, one study found a greater net decrease of Hba1c levels with doses of semaglutide up to 0.3 mg/day; however, greater gastrointestinal complaints were observed with semaglutide than liraglutide (Lingvay et al., 2018).

Additionally, another study showed that semaglutide had a decrease in HbA1c levels of 1.64% at a dosage of 1 mg, compared to 1.47% of Liraglutide at a dosage of 1.2 mg. In addition to being more effective at lowering HbA1c levels, semaglutide was also associated with greater weight loss than liraglutide, resulting in an average weight loss of 6.8-9.4 kg over 26 weeks, while people who took liraglutide lost an average of 3.8- 5.8 kg (Trujillo, Nuffer, & Smith, 2021).

Another study found that semaglutide was 0.5% more effective at reducing HbA1c levels than liraglutide, both giving a reduction of 1.8% and 1.3%, respectively. Additionally, semaglutide also showed a 3.5% more mean reduction in body weight from baseline than liraglutide (Nauck, Quast, Wefers, & Meier, 2021). However, it should be noted that valid results may not be drawn from just a few studies; therefore, it would be important to analyse a variety of sources to determine efficacy of both drugs. Examining the side effects of both drugs, both seem to have similar side effects, such as gastrointestinal complaints, chills, joint pain, and loss of appetite (Mayo Clinic, 2023).

Overall, even though they share mechanisms, semaglutide may prove slightly more beneficial for patients who are looking to lose weight and prevent future gastrointestinal complaints more associated with Liraglutide use (Malkin, Russel-Szymczyk, Liidemann, Volke, & Hunt, 2019).

**INSULIN**

Insulin is a hormone, usually homeostatically released by the body after eating to bring down blood sugar levels and absorb glucose into cells. However, in the case of type 2 diabetes, cells stop or become less responsive to insulin. In other cases, the pancreas may produce non-functional insulin. Eventually, insulin therapy may need to be started to prevent microvascular complications, like damage to nerves, leading to loss of feeling in the feet (Mayo Clinic Staff, 2023).

However, insulin and semaglutide both have different mechanisms of action. The main difference is that insulin therapy introduces synthetic insulin, while semaglutide stimulates the body's own production of insulin.

Insulin works by binding to the alpha subunit of the insulin receptor, which is a glycoprotein on the cell surface. This binding causes a conformational change, which activates the beta subunits. These activated subunits use ATP to add phosphate groups to themselves. This triggers a signalling pathway that regulates metabolism, including the storage of glucose as glycogen (UpToDate editors, 2023). To examine efficacy of insulin as compared to semaglutide, a study was done which showed insulin to be less effective at reducing HbA1c levels compared to semaglutide, showing a decrease of 1.64% for 1 mg, and 1.38% for 0.5 mg of semaglutide, but a decrease of 0.83% for insulin at both of these tested doses. In addition, insulin resulted in higher instances of hypoglycaemia than semaglutide (Aroda et al., 2017). Another study showed semaglutide was associated with greater weight loss compared to basal-bolus insulin, resulting in a range of 6.8-9.4 kg weight loss, in addition to a 0.36% decrease in HbA1c levels in patients who had a combined therapy of both insulin and semaglutide (Lingvay et al., 2023).

In other instances, semaglutide was added to regimens of patients already receiving insulin therapy, and only the effects of semaglutide were measured (Rodbard et al., 2018; Wright & Aroda, 2020). Therefore, these studies could not be used to evaluate efficacy of semaglutide against insulin therapy.

In previous trials where researchers conducted a meta-analysis of current knowledge about GLP-1 receptor agonists, semaglutide showed greater weight loss than insulin therapy, which led to a 1.15 kg weight gain with insulin, whereas a 5.17 kg weight loss was noted for the same dosage of insulin (Nauck, Quast, Wefers, & Meier, 2021). Insulin therapy may cause weight gain due to excess glucose entering cells than needed, resulting in glucose being stored as fat.

Another factor to consider is cost and availability. Insulin is widely available in

healthcare and has been in use for treatment of type 2 diabetes for several years. However, semaglutide is a very recent drug implemented into the regimens of patients. It is not as widely available as insulin around the world, being abundant in mainly western countries. As for cost, both insulin and semaglutide can be extremely expensive. However, it is much more likely that insulin therapy, not semaglutide, is covered by insurance, making it a much more cost-effective solution for patients to implement. In one study, semaglutide was found to have an average direct cost of £800 higher (around $1080) than insulin, part of which was offset due to lower diabetes related complications (Evans et al., 2023).

In conclusion, semaglutide may be a more effective treatment for type 2 diabetes compared to insulin. However, it may not suit patients who live in eastern countries or lower income patients due to semaglutide's high costs.

**SITAGLIPTIN**

Sitagliptin belongs to a class of medications known as DPP-4 inhibitors. It works by slowing down inactivation of incretins, a group of hormones that work to decrease blood glucose levels. This mechanism increases insulin secretion and decreases glucagon secretion, which is dependent on glucose homeostasis. As a result, the drug causes decreased HbA1c levels (DrugBank, n.d.).

Sitagliptin was found to be less effective at weight loss than semaglutide, showing a 6.4% decrease compared to a 15.8% in semaglutide at the same dosage. However, this study was conducted on patients without diabetes (Rubino et al., 2022). In another study, semaglutide was also found to have a greater reduction in HbA1c levels, with a treatment difference of 0.17% (Alsugair et al., 2021).

Sitagliptin and other DPP4 inhibitors are associated with a few cases of bullous pemphigoid (BP): a skin disease associated with blisters and eczema-like lesions. However, the frequency of DPP4 associated with BP was found to be around 0.00859%, making it extremely rare (Alsugair et al., 2021). On the other hand, semaglutide is associated with milder side effects, such as nausea, vomiting and other gastrointestinal complaints.

As for cost, a Swedish study found that semaglutide was found to be far more expensive than sitagliptin, costing around $22,300 per quality adjusted life year (QALY) compared to $11,200 per QALY for sitagliptin (Eliasson et al., 2022). QALY is the academic standard for measuring how well all distinct kinds of medical treatments may improve quality of lives (ICER, 2024). These prices also do not take insurance into account. It was concluded, however, that semaglutide was more cost efficient, as it was a better value for the money. This was concluded by a base-case analysis, and the robustness was evaluated with deterministic and probabilistic sensitivity analysis (Eliasson et al., 2022). Again, it is also important to note that these costs may not be representative of the global costs of sitagliptin. For instance, Swedish prices of sitagliptin or semaglutide may be cheaper than the rest of the world per year. This is significant, as Sweden is known as one of the most expensive countries in Europe.

**EMPAGLIFLOZIN**

Empagliflozin belongs to a class of medication known as SGLT-2 inhibitors. It works by reducing the re-uptake of glucose by the kidney tubules and increasing urinary excretion of glucose through the urine. Additionally, it also reduces sodium load due to its diuretic properties (meaning that it helps increase production of urine) (Sizar, Podder, & Talati, 2023).

Semaglutide provided a larger reduction in HbA1c levels, with levels being reduced by 1.3% for semaglutide, but 0.9% for empagliflozin for the same oral dosage of 14 mg, with the study being conducted over 52 weeks as a randomized clinical trial. Semaglutide also proved superior in terms of weight loss, with patients losing 0.9 kg more with semaglutide (Rodbard et al., 2019). Additionally, another meta-analysis showed that semaglutide was superior in reducing both weight and HbA1c levels, with patients losing an average of 1.65 kg more on semaglutide and reducing A1c levels by 0.61% more (Lingvay et al., 2020). It should however be noted that in both of these studies, patients were also on metformin monotherapy, which may have reduced change in weight and

HbA1c levels. Furthermore, empagliflozin has been associated with an extended lifetime, with the estimated life expectancy at patients aged 45 years was 32.1 with empagliflozin, but only 27.6 years with placebo. With confidence intervals considered, it was estimated that life expectancy would be increased by 1-5 years with empagliflozin for patients with established heart disease (Bhatt et al., 2018). As for semaglutide, a mean life span increase of 1.7 years without any cardiovascular disease was estimated, meaning that increase in lifespans may overlap for both medications – therefore, both drugs are correlated with an extended lifetime in patients.

To consider cost: semaglutide was shown to cost an average of $1100 per month compared to empagliflozin, which was shown to cost around $635 per month, making it a more affordable option than semaglutide (Vuong, 2023; Drugs.com, n.d.).

It is also important to note that instances of urinary tract infections and yeast infections have been linked to empagliflozin, with average incidences of 4.2% for 10 mg doses of the medication. Any urinary tract or yeast infections were observed 4% more often in women than men (Unnikrishnan, Kalra, Purandare, & Vasnawala, 2018).

In conclusion, semaglutide was shown to be more effective at reducing blood sugar levels and weight than empagliflozin. However, due to the association with urinary tract or yeast infections, the medication may not be suitable for patients with a history of any genital infections. Again, when prescribing medications, physicians may also need to take the financial capability of a patient into account. Therefore, semaglutide may be unsuitable for lower income patients, especially without insurance coverage.

## SOCIAL EFFECTS OF SEMAGLUTIDE: AN OUTLINE

Semaglutide is more popularly known by its brand names, Ozempic and Wegovy, both developed by the Swedish Novo Nordisk. However, given its benefits in terms of weight loss, Ozempic has especially been the centre of several discussions on social media platforms, with several celebrities also sharing their experiences online—some denying their claims of using the drug for weight loss purposes, and others voicing their opinions on the benefits and dangers of the drug.

Both Wegovy and Ozempic have been approved by the US FDA, but for different purposes: Ozempic as a treatment to lower glycated haemoglobin levels, and Wegovy as a weight loss drug—essentially a higher dosage of semaglutide. This section of the article will explore the social effects that semaglutide has had on the medical, celebrity and civilian industries.

## CELEBRITY ENDORSEMENT AND PUBLIC PERCEPTION

Ozempic has made a significant impact on social media platforms, with patient testimonials, advertisements, and even debates among doctors about the drug's benefits and risks. However, what stands out the most are the celebrity rumors that circulate online. For example, Elon Musk confirmed using Ozempic in a 2022 reply on X (formerly Twitter) while Kim Kardashian has faced ongoing speculation about using the drug to fit into a dress for the annual Met Gala—a high-profile fundraising event celebrating fashion. The SKIMS founder has repeatedly denied these claims (Head, n.d.). However, several medical professionals have taken to social media sites to help stop the spread of misinformation about the ‘miracle’ drug, many addressing that the drug could be harmful if put into the wrong hands. For instance, Dr. Mikhail Varshavski, a family medicine physician, says that many people are using Ozempic for off-brand weight loss purposes and not in the titration method (slowly increasing the dose over time) as recommended, which could worsen side effects and often has the opposite intended effect. Additionally, he touched on the drug not being the ‘miracle’ drug the public expected, with its unsupervised misuse increasing side effects, and often having extremely taxing effects on one’s mental health (Varshavski, 2023). In addition, Abbey Sharp, a registered dietitian, has also advised that “these weight loss drugs are not magic, and they still require a calorie deficit to work,” in addition to claiming that “medicine is all about risk and benefit,” suggesting that individuals should carefully consider the risk-benefit analysis to start taking

Ozempic (Abbey's Kitchen, n.d.). Even though these professionals, and many others, have helped combat misinformation and misuse, celebrity endorsement has painted a picture of the drug being somewhat of a quick fix. Sharp mentions that "this is not to help you fit into your wedding dress. This is nutrition medical therapy for a chronic disease intended to be utilized for life."

On the other hand, others are sharing their success stories all over media platforms. One woman highlights the positive impact that Ozempic had on her, quoting that "hikes that were punishing a few years ago felt easy this summer," and that she "weighed less than she did in high school." However, nothing about side effects was mentioned in this story (Marcus, 2023). Another review also mentioned mild, commonly reported side effects that cleared up within 2 weeks, quoting "the drug feels too good to be true" (Zell, 2021). These reviews are just a few examples of the hundreds of thousands of reviews circling social media sites all over the world. It is important to note that many of these reviews have been extremely contrasting, with some experiencing debilitating nausea and inability to eat at all, and others having the "best feeling of their lives." This reveals that the reviews left on online sites may be skewed, or that semaglutide truly has varying effects from person to person. Semaglutide has clearly reached far and wide, contributing to a tapestry of experiences and reviews that continue to influence public perception.

**IMPACT ON DIABETICS AND THE UNINSURED**

With a surging demand for the drug, Novo Nordisk saw an increase of 26% in their annual sales from 2021, making over $25 billion in net sales in 2022 (Novo Nordisk, 2022). However, the increase in sales was so large that diabetics began to suffer a shortage of the medication they desperately needed to survive. This may have increased their risk of heart diseases and diabetic ketoacidosis. Another problem this caused was differential pricing at the international level, with the United States having much higher prices of Ozempic per pen than Denmark ($934 in the US, versus $770 in Denmark) (Novo Nordisk, 2022; Apoteket.dk, n.d.). With the United States having one of the highest global rates of obesity and diabetes, this differential pricing has made affording the drug extremely difficult for the uninsured. The effect of the shortage on diabetics was clearly seen all over the world, with the Australian government advising doctors to "continue to consider alternatives to semaglutide until supply is expected to stabilise after 31 December 2023" (Therapeutic Goods Administration [TGA], 2023). The US FDA has also placed both Ozempic and Wegovy on its shortage database, only adding to the complexity of the issue, and leaving diabetics and the uninsured in uncertainty about their health outcomes (FDA Drug Shortages, n.d.).

Additionally, this shortage has left many questioning whether this is a symptom of inequality, laying bare already existing disparities in the healthcare system. For instance, Black and Hispanic patients in the USA were found to historically have poorer health outcomes than white patients (24.9% of the Black population was found to be in poor or fair health, as compared to 6.3% of the white population) (Mahajan et al., 2021). A larger proportion of the Black population that used semaglutide faced the most barriers in affording its injections, with the proportion of Black adults not covered under insurance being around 16.4% compared to 8.7% for white eligible adults (Lu, Liu, & Krumholz, 2022). This highlights the shocking discrepancy between socio-economic factors and how they intertwine with access to certain medications.

**POTENTIAL FUTURE DEVELOPMENTS AND ALTERNATIVES**

Due to the semaglutide shortage, the general population has started to seek alternatives that have similar effects and are more widely available to help speed up their weight loss journey. In this section, we will cover newer medications and future alternatives for the treatment of type 2 diabetes.

In recent months, a new drug seems to have taken over the diabetes market: Mounjaro. It contains the active ingredient tirzepatide, a glucose-dependent GIP/GLP-1 receptor co-agonist. This means it acts as an incretin hormone, triggering the same pathway as semaglutide. Tirzepatide has been shown to improve insulin sensitivity and delay gastric emptying, both of which contribute to weight loss, much like semaglutide (DrugBank, n.d.). Tirzepatide is different from semaglutide because it acts as a dual receptor agonist—making it more effective than semaglutide in many cases, as it targets the gastric inhibitory polypeptide (or

GIP), which improves insulin secretion, enhances fat metabolism, and helps regulate appetite as well as GLP-1. For instance, tirzepatide proved superior to semaglutide, decreasing HbA1c levels by 1.86 percentage points than semaglutide at the 5 mg dose (Juan et al., 2021). Additionally, it was shown to decrease weight by about 17% of the mean body weight, compared to 12.4% with semaglutide. It was also shown to be a cheaper alternative, with the estimated cost being around $860 lower than semaglutide per 1% of weight lost (Azuri et al., 2023). This reduction in cost and higher efficacy may be what has caused "Ozempic to take a back seat," according to Dr. Seshadri, an internal medicine physician practicing in Abu Dhabi.

In addition, there are several future treatments to diabetes that have yet to be fully put into use. For instance, modifying gut flora to prevent diabetic complications such as diabetic nephropathy. It has been shown that diabetic patients often have gut dysbiosis: an imbalance in the bacteria that reside in the gut of humans. This problem can potentially lead to more insulin resistance, chronic inflammation, and obesity: problems that all contribute to the development of type 2 diabetes. Additionally, the composition of these bacteria has been shown to be different in patients of type 2 diabetes, implying that they may have a role to play in the pathophysiology of type 2 diabetes (Zheng, Bao, Dongsheng, & Chunsheng, 2022). One study showed that a microbiota transplant from healthy lean donors significantly reduced their insulin resistance, and that this was dependent on higher diversity of gut flora than baseline (Kootte et al., 2017). Overall, this evidence shows promise in treating patients' insulin sensitivity, and ultimately, type 2 diabetes.

Another promising treatment is stem cell transplants of beta cells. These stem cells can come from a variety of sources, including pluripotent, multipotent, and mesenchymal stem cells. The first two types of cells can be sourced from leftover embryos from in-vitro fertilisation (IVF) or be programmed from adult cells. This is particularly useful, as it can create cells specific to the patient and reduce chances of rejection (Takahashi & Yamanaka, 2006). One animal study showed reversal of diabetes in mice within 40 days with the use of embryonic stem cells, proving more effective than previously used multipotent stem cells sourced from the innermost layer of the gut (Rezania et al., 2014). This could be because of the insulin-producing beta cells being regenerated in mice, allowing for improved blood sugar control.

Furthermore, a contact lens that can sense blood glucose level has been in development. This revolutionary method is not the first wearable technology, though, as automated insulin pumps are widely used for monitoring. However, with it being so portable, minimally invasive, and a viable option, it could have massive impacts and potential in the world of diabetes treatment (Kim et al., 2022). It works through sensing glucose levels in tears through fluorescent glucose sensors (Mohamed et al., 2022). For instance, changes in glucose levels would turn the lens from pink to blue. This evolutionary device may provide a more discreet and continuous monitoring solution for patients to consider in their diabetes treatment.

**CONCLUSION**

In conclusion, semaglutide has proven itself to be a multifaceted drug with significant implications in both clinical and social contexts. Its mechanism as a GLP-1 receptor agonist allows it to effectively reduce HbA1c levels, promote weight loss, and improve cardiovascular outcomes in patients with type 2 diabetes. When compared to other medications across different classes—including metformin, insulin, liraglutide, sitagliptin, and empagliflozin—semaglutide generally seems to offer superior glycemic control and weight-related benefits, although its cost and limited paediatric application present important limitations.

Socially, the drug's rise in popularity, fueled by celebrity endorsements and media exposure, has sparked widespread off-label use, shifting public belief and complicating access for diabetic patients due to increased demand and global shortages. These issues have also magnified pre-existing disparities in healthcare access, especially among low-income and uninsured populations. Finally, while newer alternatives like tirzepatide and future innovations such as microbiome therapy and stem cell research show promise, semaglutide remains a powerful, though controversial, tool

in modern diabetes care. Ultimately, a balanced view—grounded in evidence, ethical considerations, and equitable access—is needed to navigate semaglutide's double role as a medical treatment and social phenomenon.

**REFERENCES**


1. Abbey's Kitchen. (n.d.). The Truth About Ozempic: A Dietitian's Perspective.

2. Administration, T. G. (2025, April 9). About the Ozempic (semaglutide) shortage. Therapeutic Goods Administration (TGA).

3. Alsugair HA, Alshugair IF, Alharbi TJ, Bin Rsheed AM, Tourkmani AM, Al-Madani W. Weekly Semaglutide vs. Liraglutide Efficacy Profile: A Network Meta-Analysis. Healthcare (Basel). 2021 Aug 30;9(9):1125.

4. Apoteket.dk.Ozempic®.

5. Aroda VR, Bain SC, Cariou B, Piletič M, Rose L, Axelsen M, Rowe E, DeVries JH. Efficacy and safety of once-weekly semaglutide versus once-daily insulin glargine as add-on to metformin (with or without sulfonylureas) in insulin-naive patients with type 2 diabetes (SUSTAIN 4): a randomised, open-label, parallel-group, multicentre, multinational, phase 3a trial. Lancet Diabetes Endocrinol. 2017 May.

6. Aroda VR, Bauer R, Christiansen E, Haluzík M, Kallenbach K, Montanya E, Rosenstock J, Meier JJ. Efficacy and safety of oral semaglutide by subgroups of patient characteristics in the PIONEER phase 3 programme. Diabetes Obes Metab. 2022 Jul.

7. Azuri J, Hammerman A, Aboalhasan E, Sluckis B, Arbel R. Tirzepatide versus semaglutide for weight loss in patients with type 2 diabetes mellitus: A value for money analysis. Diabetes Obes Metab. 2023 Apr.

8. Bhatt, D. L., Steg, P. G., Emdin, C. A., James, S. K., Živkovic, G., Chin, K. H., ... & Desai, A. K. (2018). Long-term benefit of empagliflozin on life expectancy in patients with type 2 diabetes mellitus and established cardiovascular disease.

9. Blough B, Moreland A, Mora A Jr. Metformin-induced lactic acidosis with emphasis on the anion gap. Proc (Bayl Univ Med Cent). 2015 Jan 28.

10. Buse JB, Bode BW, Mertens A, et al. Long-term efficacy and safety of oral semaglutide and the effect of switching from sitagliptin to oral semaglutide in patients with type 2 diabetes: a 52- week, randomized, open-label extension of the PIONEER 7 trial.

11. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. Metabolism. 2016 Feb.

12. Drugbank, Tirzepatide.

13. DrugBank. (n.d.). Metformin.

14. DrugBank. Sitagliptin.

15. Drugs.com. Jardiance price guide.

16. E Mohamed, M Rosalia, A Fahad, S Ahmed E., A Israr, B Haider "Wearable Smart Contact Lenses for Continual Glucose Monitoring: A Review" Frontiers in Medicine Volume 9 2022.

17. Eliasson B, Ericsson Å, Fridhammar A, Nilsson A, Persson S, Chubb B. Long-Term Cost Effectiveness of Oral Semaglutide Versus Empagliflozin and Sitagliptin for the Treatment of Type 2 Diabetes in the Swedish Setting. Pharmacoecon Open. 2022 May.

18. Eugene E. Wright Jr & Vanita R. Aroda (2020) Clinical review of the efficacy and safety of oral semaglutide in patients with type 2 diabetes considered for injectable GLP-1 receptor agonist therapy or currently on insulin therapy, Postgraduate Medicine.

19. Evans M, Chubb B, Malkin SJP, Berry S, Lawson J, Hunt B. Once-weekly semaglutide versus insulin aspart for the treatment of type 2 diabetes in the UK: A long-term cost-effectiveness analysis based on SUSTAIN 11. Diabetes Obes Metab. 2023 Feb;25(2):491-500.

20. FDA approves once-weekly Wegovy injection for the treatment of obesity in teens aged 12 years and older. Novo Nordisk USA. December 23, 2022.

21. FDA drug shortages (no date) FDA Drug Shortages.

22. Ghusn W, De la Rosa A, Sacoto D, Cifuentes L, Campos A, Feris F, Hurtado MD, Acosta A. Weight Loss Outcomes Associated With Semaglutide Treatment for Patients With Overweight or Obesity. JAMA Netw Open. 2022 Sep 1.

23. Head. A, 'A diabetes drug is going viral for its supposed weight loss benefits—but there's a seriously dark problem here'.

24. Helena W Rodbard et al, Semaglutide Added to Basal Insulin in Type 2 Diabetes (SUSTAIN 5): A Randomized, Controlled Trial, The Journal of Clinical Endocrinology & Metabolism, Volume 103, Issue 6, June 2018, Pages 2291–2301.

25. Helena W. Rodbard, Julio Rosenstock, Luis H. Canani, Chaicharn Deerochanawong, Janusz Gumprecht, Søren Østergaard Lindberg, Ildiko Lingvay, Anette Luther Søndergaard, Marianne Bach Treppendahl, Eduard Montanya, PIONEER 2 Investigators; Oral Semaglutide Versus Empagliflozin in Patients With Type 2 Diabetes Uncontrolled on Metformin: The PIONEER 2 Trial. Diabetes Care 1 December 2019.

26. https://twitter.com/elonmusk/status/1576367983051489281.

27. https://www.marieclaire.co.uk/life/health-fitness/ozempic-for-weight-loss.

28. Ildiko Lingvay and others, Efficacy of Once-Weekly Semaglutide vs Empagliflozin Added to Metformin in Type 2 Diabetes: Patient-Level Meta-analysis, The Journal of Clinical Endocrinology & Metabolism, Volume 105, Issue 12, December 2020, Pages e4593–e4604.

29. Ildiko Lingvay, Cyrus V. Desouza, Katarina S. Lalic, Ludger Rose, Thomas Hansen, Jeppe Zacho, Thomas R. Pieber; A 26-Week Randomized Controlled Trial of Semaglutide Once Daily Versus Liraglutide and Placebo in Patients With Type 2 Diabetes Suboptimally Controlled on Diet and Exercise With or Without Metformin. Diabetes Care 1 September 2018.

30. Juan P. and Davies, Melanie J. and Rosenstock, Julio and Perez Manghi, Federico C. Fernandez Lando Laura and Bergman, Brandon K. and Liu, Bing and Cui, Xuewei and Brown, Katelyn.

31. Juan Pang, Jia Nuo Feng, Wenhua Ling, Tianru Jin, The anti-inflammatory feature of glucagon-like peptide-1 and its based diabetes drugs—Therapeutic potential exploration in lung injury, Acta Pharmaceutica Sinica B, Volume 12, Issue 11, 2022.

32. K Poorva, Interview with Dr Sunil Sheshadri – internal medicine physician, date of interview: 27/08/2023

33. Kalra, Sanjay & Sahay, Rakesh. (2020). A Review on Semaglutide: An Oral Glucagon-Like Peptide 1 Receptor Agonist in Management of Type 2 Diabetes Mellitus. Diabetes Therapy.

34. Khang T. Vuong (2023, March 8). Semaglutide drugs Rybelsus, Ozempic, Wegovy cost with & without insurance?.

35. Kim SK, Lee GH, Jeon C, Han HH, Kim SJ, Mok JW, Joo CK, Shin S, Sim JY, Myung D, Bao Z, Hahn SK. Bimetallic Nanocatalysts Immobilized in Nanoporous Hydrogels for Long-Term Robust Continuous Glucose Monitoring of Smart Contact Lens. AdvMater. 2022 May.

36. Klec C, Ziomek G, Pichler M, Malli R, Graier WF. Calcium Signaling in ß-cell Physiology and Pathology: A Revisit. Int J Mol Sci. 2019 Dec.

37. Kolkailah, AA, Lingvay, I, Dobrecky-Mery, I, et al. Effects of once-weekly subcutaneous semaglutide on coronary artery disease outcomes in patients with type 2 diabetes with or at high risk for cardiovascular disease: Insights from the SUSTAIN-6 trial. Diabetes Obes Metab. 2023.

38. Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra AV, Udayappan SD, Hermes G, Bouter KE, Koopen AM, Holst JJ, Knop FK, Blaak EE, Zhao J, Smidt H, Harms AC, Hankemeijer T, Bergman JJGHM, Romijn HA, Schaap FG, Olde Damink SWM, Ackermans MT, Dallinga-Thie GM, Zoetendal E, de Vos WM, Serlie MJ, Stroes ESG, Groen AK, Nieuwdorp M. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. Cell Metab.

39. Lingvay I, Catarig AM, Lawson J, Chubb B, Gorst-Rasmussen A, Evans LM. An Indirect Comparison of Basal Insulin Plus Once-Weekly Semaglutide and Fully Optimised Basal-Bolus Insulin in Type 2 Diabetes. Diabetes Ther. 2023 Jan.

40. Lu, Y., Liu, Y., & Krumholz, H. M. (2022). Racial and Ethnic Disparities in Financial Barriers Among Overweight and Obese Adults Eligible for Semaglutide in the United States. Journal of the American Heart Association.

41. Mahajan S, Caraballo C, Lu Y, Valero-Elizondo J, Massey D, Annapureddy AR, Roy B, Riley C, Murugiah K, Onuma O, Nunez-Smith M, Forman HP, Nasir K, Herrin J, Krumholz HM. Trends in Differences in Health Status and Health Care Access and Affordability by Race and Ethnicity in the United States, 1999-2018. JAMA. 2021 Aug 17.

42. Mahapatra MK, Karuppasamy M, Sahoo BM. Semaglutide, a glucagon like peptide-1 receptor agonist with cardiovascular benefits for management of type 2 diabetes. Rev Endocr Metab Disord. 2022 Jun.

43. Marcus, R. (2023, June 6). Ozempic and Weight Loss. The Washington Post.

44. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T; SUSTAIN-6 Investigators. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med. 2016 Nov 10.

45. Mayo Clinic Staff. (2023, February 1). Diabetes Treatment: In Depth.

46. Mayo Clinic. (2023, February 1). Liraglutide (Subcutaneous Route) Side Effects.

47. Meier JJ. Efficacy of Semaglutide in a Subcutaneous and an Oral Formulation. Front Endocrinol (Lausanne). 2021 Jun 25.

48. N Traaseth, S Elfering, J Solien, V Haynes, C Giulivi, Role of calcium signaling in the activation of mitochondrial nitric oxide synthase and citric acid cycle, Biochimica et Biophysica Acta (BBA) - Bioenergetics, Volume 1658, Issues 1–2,2004, Pages 64-71, ISSN 0005-2728.

49. Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. Mol Metab. 2021 Apr.

50. Novo Nordisk, December 2022 [annual report 2022].

51. Novo Nordisk, “Ozempic Australian product information November 2020”.

52. Novo Nordisk. Ozempic® (semaglutide) injection 0.5 mg, 1 mg, 2 mg List Price & Insurance Coverage Explained | NovoCare®.

53. Pharmacoeconomic Review Report: Semaglutide (Ozempic): (Novo Nordisk Canada Inc.): Indication: For the treatment of adults patients with type 2 diabetes mellitus to improve glycemic control, in combination with metformin (second-line treatment), and in combination with metformin and sulfonylurea (third-line treatment) [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2019 Jun.

54. Practice Committee of the American Society for Reproductive Medicine. Practice Committee of the American Society for Reproductive Medicine. Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. Fertil Steril. 2017 Sep.

55. Prasad F, De R, Korann V, et al. Semaglutide for the treatment of antipsychotic-associated weight gain in patients not responding to metformin – a case series. Therapeutic Advances in Psychopharmacology. 2023.

56. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, Yang YH, Johnson JD, Kieffer TJ. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014 Nov.

57. Rix I, Nexøe-Larsen C, Bergmann NC, et al. Glucagon Physiology. [Updated 2019 Jul 16]. In: Feingold KR, Anawalt B, Blackman MR, et al.,

editors. Endotext [Internet]. South Dartmouth (MA).

58. Rogers JK, Yaroshinsky A, Pocock SJ, Stokar D, Pogoda J. Analysis of recurrent events with an associated informative dropout time: Application of the joint frailty model. Stat Med. 2016.

59. Rubino DM, Greenway FL, Khalid U, et al. Effect of Weekly Subcutaneous Semaglutide vs Daily Liraglutide on Body Weight in Adults With Overweight or Obesity Without Diabetes: The STEP 8 Randomized Clinical Trial. JAMA. 2022.

60. Ruder K. As Semaglutide's Popularity Soars, Rare but Serious Adverse Effects Are Emerging. JAMA.

61. Sizar O, Podder V, Talati R. Empagliflozin. [Updated 2023 May 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan.

62. Soliman A, De Sanctis V, Alaaraj N, Hamed N. The clinical application of metformin in children and adolescents: A short update. Acta Biomed. 2020 Sep 7.

63. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25.

64. Tasanen K, Varpuluoma O, Nishie W. Dipeptidyl Peptidase-4 Inhibitor-Associated Bullous Pemphigoid. Front Immunol. 2019 Jun.

65. Taylor SI. The High Cost of Diabetes Drugs: Disparate Impact on the Most Vulnerable Patients. Diabetes Care. 2020 Oct.

66. Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes, New England Journal of Medicine.

67. Unnikrishnan AG, Kalra S, Purandare V, Vasnawala H. Genital Infections with Sodium Glucose Cotransporter-2 Inhibitors: Occurrence and Management in Patients with Type 2 Diabetes Mellitus. Indian J Endocrinol Metab. 2018 Nov-Dec.

68. UpToDate editors. (2023). Insulin Action.

69. Varshavski, M. (2023, February 14). How to lose weight and keep it off [Video]. YouTube.

70. Vilsbøll T, Lindahl CØ, Nielsen NF, Tikkanen CK. Real-world impact of once-weekly subcutaneous semaglutide after 2 years of follow-up: Results from a nationwide observational study in people with type 2 diabetes. Diabetes Obes Metab. 2023 Jun.

71. Wadden TA, Bailey TS, Billings LK, Davies M, Frias JP, Koroleva A, Lingvay I, O'Neil PM, Rubino DM, Skovgaard D, Wallenstein SOR, Garvey WT; STEP 3 Investigators. Effect of Subcutaneous Semaglutide vs Placebo as an Adjunct to Intensive Behavioral Therapy on Body Weight in Adults With Overweight or Obesity: The STEP 3 Randomized Clinical Trial. JAMA. 2021 Apr.

72. Wegovy® Dosing Schedule | Wegovy® (semaglutide) Injection 2.4 mg. (n.d.). https://www.wegovy.com/taking-wegovy/dosing-schedule.html

73. Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, McGowan BM, Rosenstock J, Tran MTD, Wadden TA, Wharton S, Yokote K, Zeuthen N, Kushner RF; STEP 1 Study Group. Once-Weekly Semaglutide in Adults with Overweight or Obesity. N Engl J Med. 2021 Mar.

74. Zell, (2021, March 19) [review about Ozempic].

75. Zhao X, Wang M, Wen Z, Lu Z, Cui L, Fu C, Xue H, Liu Y and Zhang Y (2021) GLP-1 Receptor Agonists: Beyond Their Pancreatic Effects. Front. Endocrinol. 12:721135.

76. Zheng.Z, Bao.S, Dongsheng.Y, Chunsheng.Z Gut Microbiota: An Important Player in Type 2 Diabetes Mellitus Frontiers in Cellular and Infection Microbiology VOLUME 12 2022.

# AN INVESTIGATION ON THE ALLEVIATION AND REPAIR EFFECTS OF *BACILLUS PUMILUS* LV-149 SOLUTIONS AT DIFFERENT CONCENTRATIONS ON CELLULAR ULCERATIVE COLITIS IN MICE COLON CELLS OVER DSS SOLUTION - INDUCED TREATMENT

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**ABSTRACT**
*Bacillus pumilus* (*B. pumilus*) is a facultative, gram-positive probiotic bacterium with immunomodulatory, anti-inflammatory, and antimicrobial properties. Previous studies have demonstrated its ability to inhibit pathogenic bacteria, promote intestinal health, and serve as a biological control agent. However, its potential role in alleviating and repairing inflammatory damage in ulcerative colitis (UC) remains under-explored. UC is a chronic inflammatory bowel disease characterized by disruption of the intestinal barrier, increased intestinal permeability, excessive production of pro-inflammatory cytokines, and decreased levels of tight-junction proteins. This study investigates the effects of different concentrations of *B. pumilus* LV-149 ($1\times10^8$ CFU/mL and $1\times10^9$ CFU/mL) on UC-induced mice models treated with 3% DSS solution. Key inflammatory markers, including pro-inflammatory cytokines (IL-6, IL-1β), anti-inflammatory cytokine (IL-10), and tight-junction protein claudin-1, are measured at the gene expression level using real-time fluorescence quantitative polymerase chain reaction (qRT-PCR). The study aims to determine whether *B. pumilus* can mitigate intestinal inflammation by regulating cytokine production and restoring tight-junction integrity. By evaluating the impact of *B. pumilus* on the intestinal barrier and inflammatory response, this research provides new insights into its potential as a probiotic therapeutic for UC treatment. Findings from this study may contribute to the development of novel probiotic-based interventions for inflammatory bowel diseases, offering a safe and effective alternative to conventional treatments.

## BACKGROUND

### THE INTESTINAL BARRIER AND ULCERATIVE COLITIS

Mechanical barriers are the first line of defense for substances harmful to human health, effectively inhibiting the entry of pathogenic substances into the bloodstream (Fahey et al., 2018). The chemical barrier is mainly composed of antibacterial substances secreted by intestinal microorganisms, mucins and antimicrobial peptides secreted by intestinal epithelial cells, which can prevent intestinal contents from damaging the upper skin cells (Johansson et al., 2012). The intestinal permeability increases the severity of colitis by interacting with the inflammatory response (Kitajima et al.). The intestinal barrier in UC patients is destroyed, causing immune response and releasing a large number of inflammatory factors, triggering intestinal inflammation (Vancamelbeke et al., 2017). The goblet cells responsible for the mucus secretion in the intestine of UC patients are reduced, resulting in thinning or even disappearance of the mucus layer, and antigens invade exposed cells, exacerbating intestinal inflammation (Vindigni et al., 2016).

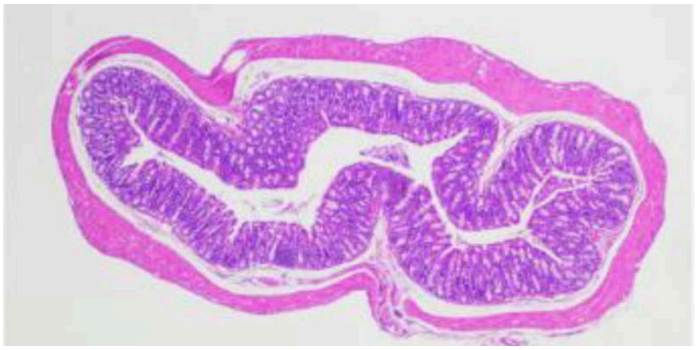


Figure 1: Mouse colon slice (Shao et al., 2021)

### *BACILLUS PUMILUS*

*B.pumilus* is a short rod-shaped, facultative, gram-positive bacterium belonging to the *Bacillus* family genus, and is considered a probiotic. Probiotics produce molecules with immunomodulatory and anti-inflammatory functions, which regulate the immune system by stimulating epithelial, lymphocytes, or dendritic cells. (D'Amelio et al., 2018).

Studies show that including probiotics in shrimp feed could effectively inhibit intestinal pathogen growth, reduce the occurrence of vibrio disease, and be possessed with high biological safety (Luo & Jiang et al., 2013). Zeng et al. found that *B. pumilus* isolated from the intestines of yaks exhibited strong antioxidant, colonization, tolerance (acid, bile salt, and heat), as well as the ability to inhibit the growth of pathogenic bacteria. Dai et al. found that *B. pumilus* is a biological control bacterium, and the protein contained in the fermentation broth of the strain can resist fungal substances. Saggese et al. found that the SF214 strain of *Bacillus pumilus* isolated from the ocean can produce two different antibiotics, which have specific antibacterial effects against *Staphylococcus aureus* and *Listeria monocytogenes*. *B. pumilus* acts against pathogenic bacteria and is safe for biological usage. However, the optimal concentration of *B. pumilus* solution for the process and repair of inflammatory tissue has not yet been explored. As a probiotic with extremely high medicinal potential, *B. pumilus* could be utilized as an effective method for treating human ulcerative colitis.

### COLONIC PRO AND ANTI-INFLAMMATORY CYTOKINES IN ULCERATIVE COLITIS

Although the etiology of UC has not been defined, numerous studies have shown that loss of anti-inflammatory factors or excessive production of pro-inflammatory factors induce the intestinal barrier structure. IL-6 is an environment-dependent multifunctional cytokine, which cannot only promote normal immunity and tissue regeneration, but can also regulate the proliferation of T cells and promote the production of other inflammatory factors (Hunter & Jones et al, 2015). IL-6 was found to play an important role in UC development, in addition to intensifying tissue inflammation by inhibiting T cell apoptosis, and also plays a repair function at the intestinal barrier (Friedrich et al, 2019). IL-1β is a typical pro-inflammatory cytokine that is crucial for the host's defense against infection and injury. IL-1β is expressed in macrophages of non-lymphoid organs such as lungs, digestive tract, and liver. Interleukin-10 (IL-10) is a multifunctional cytokine that can exert immunostimulatory effects in various types of cells. IL-10 can downregulate the expression of major histocompatibility antigen II on the surface of monocytes, and reduce its antigen presentation effect.

## TIGHT JUNCTION PROTEINS IN ULCERATIVE COLITIS

Tight junction proteins (occludin, claudin, ZOs, etc.) are important components of the intestinal barrier. The content of claudins affects the formation of tight junction bands and the ion-selective permeability of intestinal epithelium. These proteins are connected to the epithelial cytoskeleton by actin and myosin and play key roles in tissue differentiation, maintaining intestinal permeability and homeostasis, and preventing invasion of enteric pathogens and toxins into the colonic barrier (Chen & Zhang et al., 2019). A study by Kitajima et al. states that, in DSS induced UC mice, the colonic mucosa did not show inflammatory cells initially, the colonic epithelial cells were shed later, and the expression of tight junction protein was reduced, leading to bacteria entering the intestinal mucosa and eventually an inflammatory response. Tan et al. found that abnormal expression of tight junction proteins caused intestinal mucosa to heal, and had a higher degree of mucosal healing. Therefore, the expression of the tight junction proteins may be an important indicator to evaluate the mucosal healing in UC.

## DISUCCINIMIDYL SUBERATE INDUCED UC COLON CELLS

Antibodies were cross-linked to Protein A or G agarose beads using disuccinimidyl suberate (DSS, Fig. 2), a bifunctional cross-linker that reacts with two amine groups to form stable amide bonds (DeCaprio et al., 2019). A 3% DSS solution was also used to induce an inflammatory response and simulate ulcerative colitis in pre-cultured mouse colon cells.

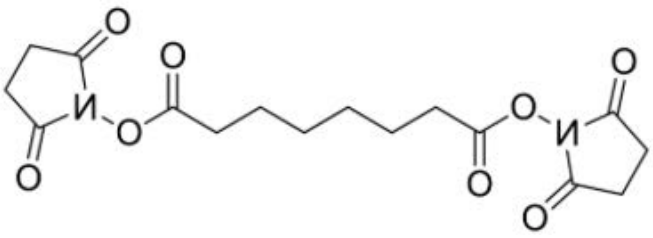


Figure 2: Chemical structure of disuccinimidyl suberate (Cayman Chemical, 2025)

## HYPOTHESIS

### ALTERNATIVE HYPOTHESIS

There will be a significant difference in the expression level of inflammatory factor genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

### NULL HYPOTHESIS

There will be no significant difference in the expression level of inflammatory factors genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

## EXPERIMENTAL DESIGN

### INDEPENDENT AND DEPENDENT VARIABLES

The independent variable in this study was the concentration of *B. pumilus* LV-149 probiotic strain solutions, which were set at $1\times10^8$ CFU/mL and $1\times10^9$ CFU/mL. These concentrations were chosen to ensure optimal therapeutic effects on colon cells. Higher concentrations of the probiotic solution were hypothesized to have a stronger ability to alleviate and repair damaged UC colon cells compared to lower concentrations. The curative effects of the probiotic treatments were measured through gene expression levels. The dependent variable was the gene expression level of inflammatory factors and tight-junction proteins. The primary factors used to evaluate the therapeutic effects of LV-149 were pro-inflammatory cytokines IL-6 and IL-1β, the anti-inflammatory cytokine IL-10, and the tight-junction protein Claudin-1. It was expected that probiotic-treated samples would exhibit increased expression levels of IL-10 and Claudin-1 while reducing IL-6 and IL-1β, thereby indicating improved intestinal barrier function and reduced inflammation.

### CONTROLLED VARIABLES

Several controlled variables were maintained throughout the experiment to ensure accuracy and consistency in results. Temperature control was essential as high temperatures could lead to the inactivation of proteases and fluorescent enzymes, potentially causing experimental failure. Repeated freezing and thawing inhibited enzyme activity and could affect the stability of the cDNA structure, ultimately influencing qPCR data. To mitigate this, solutions were prepared and RNA was extracted on a porous metal plate stored at 4°C, and reagents were frozen immediately after use. DSS processing time

was another critical factor since variations in exposure duration could lead to inconsistent inflammatory responses in the colon cells. To maintain consistency, all samples were subjected to DSS treatment for a strictly controlled period.

Similarly, the processing time of *B. pumilus* LV-149 was regulated, as prolonged exposure could influence cytokine secretion and immune protein production. The immersion period of cells in bacterial solutions was carefully controlled to ensure standardization across all experimental groups. RNA concentration consistency was also ensured, as variations in nucleic acid concentrations could lead to errors during amplification, affecting qPCR data reliability. RNA concentrations were meticulously measured using a UV-Vis spectrophotometer, and cDNA samples were thoroughly mixed with system buffers before spotting. Finally, oscillation and vortex duration were precisely controlled. Excessive vortexing could rupture cell membranes, leading to invalid qPCR amplification. To prevent this, oscillation was performed within a predetermined operational window to minimize the risk of excessive cell wall fragmentation.

## MATERIALS

*Table 1: Materials and apparatus in this study*

| Measurement Apparatus | General Apparatus | Materials |
|---|---|---|
| RT-qPCR instrument | Pipette gun | Trizol reagent |
| Invert microscope | Clean bench | Isopropanol |
| Analytic balance | Fume hood | RevertAidTM First |
| Thermostat water bath | Ultra-low temp storage | Strand cDNA synthesis kit |
| Nucleic acid protein concentration analyzer | High-pressure steam Sterilization pot | oligo(dT)18 primer |
| PCR instrument | Ultra-pure water system | Mice colon cells (bought from Shanghai Biotechnology Co., Ltd.) |
| Volumetric flask | Electric blast drying oven | Chloroform |
| Measuring cylinder | Cell culture incubator | DEPC water |
| | Cell culture plate | Cell culture medium |
| | Vortex oscillator | Ethanol (anhydrous/75%) |
| | | RNA free enzyme water |
| | | PCR tube Cooling Chamber |

*Table 2: Genetic sequence for Interleukin and reference gene primers*

| Genetic Sequence Used (F/R Primers) | | |
|---|---|---|
| **Primers** | | **Sequence** |
| **IL-1β** | **F** | GCAACTGTTCCTGAACTCAACT |
| | **R** | ATCTTTTGGGGTCCGTCAACT |
| **IL-6** | **F** | TAGTCCTTCCTACCCCAATTTCC |
| | **R** | TTGGTCCTTAGCCACTCCTTC |
| **IL-10** | **F** | GCTCTTACTGACTGGCATGAG |
| | **R** | CGCAGCTCTAGGAGCATGTG |
| **Claudin-1** | **F** | TATCATCCTGGCCGTGCTA |
| | **R** | CATCATCCACGCAGTTGGT |
| **GAPDH** | **F** | AGGTCGGTGTGAACGGATTT |
| | **R** | TGTAGACCATGTAGTTGAGGTCA |

## SAFETY, ETHICAL, AND ENVIRONMENTAL CONSIDERATIONS

*Table 3: Safety, ethical, and environmental considerations*

| | Description | Methods taken |
|---|---|---|
| **Safety** | The experiment requires the usage of multiple hazardous chemicals and biological agents, which could lead to severe health issues, like skin and eye irritation, if some agents were accidentally intaken. | (1) Wear protective eye goggles and nitrile gloves. (2) Experimental procedures should be finished within the fume hood to prevent volatile liquid escape. (3) Follow standardized procedures. |
| **Ethical considerations** | Mice colon cells are used during the experiment and treated with reagents. | The mice colon cells have been previously extracted from experimental mice and target cells have been cultivated at the biological instrument company. |
| **Environmental** | The LV149 probiotic fluids, chemicals and different kinds of enzymes during the process of reverse transcription and extraction of total RNA and RT-qPCR could pollute the environment. | The chemicals and reagents used were strictly regulated. All chemicals should be collected using a waste tank instead of pouring into the sink directly. The wasted reagents will then be treated using professional methods. |

## PROCEDURES

Initially, pre-cultured mice colon cells were prepared. These cells were randomly separated into four groups. Afterwards, the colon cells were treated with DSS solution with the concentration of 3%, while the cells were immersed into the DSS solution for 18 hours to successfully induce the UC inflammatory response. Cells were then placed in a constant temperature shaker to ensure uniform mixing of reagents with each cell, reducing the possibility of experimental errors and failures. Cells, after being treated with 3% DSS solution, were rinsed three times with 0.9% physiological saline solution. All cell suspensions were loaded into EP tubes and

centrifuged at 12000 r/min for 10 minutes. The supernatant was retained and repackaged. One portion of the retained supernatant was used to conduct the experiment, and the rest were kept at -80 ℃ for future usage.

## PREPARATION OF LIVE *B. PUMILUS* LV-149 SUSPENSION

*B. pumilus* LV-149, stored at -80°C, was inoculated into LB medium and incubated at 37°C for 12 hours to generate seed cultures. Seed culture (5% v/v) was transferred into fresh LB liquid medium and incubated at 37°C for an additional 5 hours. Bacterial cells were harvested by centrifugation at 6500g for 5 minutes at 4°C. The pellet was washed twice with 0.9% physiological saline solution (PSS) and re-suspended in PSS to achieve a final concentrations of $1\times10^8$ CFU/mL and $1\times10^9$ CFU/mL.

## DSS-INDUCED UC MODEL

Mice colon cells were pre-cultured under standard conditions. Cells were randomly divided into four experimental groups. The UC model was induced by exposing cells to a 3% DSS solution for 18 hours, ensuring an adequate inflammatory response. The cells were kept in a constant temperature shaker to maintain homogeneous reagent mixing. After DSS treatment, cells were washed three times with 0.9% physiological saline solution to remove residual DSS. The cells were then harvested, suspended in EP tubes, and centrifuged at 12,000 r/min for 10 minutes. The supernatant was retained for further experiments, while remaining samples were stored at -80°C for future use.

## RNA EXTRACTION AND RT-qPCR

Total RNA was extracted using Trizol reagent. Briefly, 100 mg of treated cells was transferred into an EP tube, followed by the addition of 1 mL Trizol. The mixture was vortexed thoroughly, and 250 µL of chloroform was added. After mixing and resting at room temperature for 3 minutes, samples were centrifuged, and the aqueous phase was transferred to a new tube. RNA was precipitated by adding 0.8× volume of isopropanol, followed by centrifugation. The pellet was washed with ethanol, dried, and re-suspended in 100 µL RNase-free water. The RNA concentration was determined using a UV-Vis spectrophotometer and standardized to 100 ng/µL before storage at -80°C.

Reverse transcription to cDNA was performed using the RevertAidTM First Strand cDNA Synthesis Kit. cDNA synthesis was carried out at 42°C for 1 hour, followed by enzyme inactivation at 70°C for 5 minutes. For qRT-PCR, reactions were prepared in a 96-well plate using SYBR Green Master Mix, gene-specific primers, and cDNA template. GAPDH was used as an internal reference gene. Each reaction was performed in triplicate. The 96-well plate was sealed with thermoplastic film, placed in a qPCR instrument, and subjected to thermal cycling and fluorescence detection.

Figure 3: Plate setup of 96-well plate containing GAPDH & inflammatory cytokine genes

## DATA COLLECTION

### QUALITATIVE DATA

There is no significant qualitative data presented, since the investigations are mainly based on micro-aspects of genes and protein levels of cells.

### QUANTITATIVE DATA

Due to the extensively large sample data, one qPCR data of Interleukin and its Ct value calculation will be retained in the article, while the rest will be presented in the Appendix section at the end of the article. The Ct (cycle threshold) value refers to the number of PCR cycles required for the fluorescence signal in each reaction tube to exceed a predefined threshold, indicating the point at which the target nucleic acid becomes detectable above background levels. Ct values correspond to initial quantities of DNA/RNA, making Ct useful for expressing relative gene expression.

*Table 4: Ct value of Interleukin-1β in mice colon cells collect from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution*

| Interleukin-1β RT-qPCR Ct Value Data | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | GAP | IL-1β | Group | GAP | IL-1β | Group | GAP | IL-1β | Group | GAP | IL-1β |
| $C_1$ | 18.97 | 29.40 | $D_1$ | 22.13 | 27.41 | $H_1$ | 20.33 | 27.95 | $L_1$ | 23.91 | 31.17 |
| | 18.49 | 28.82 | | 22.43 | 27.21 | | 20.53 | 27.96 | | 24.53 | 30.8 |
| | 18.7 | 29.75 | | 22.34 | 27.11 | | 20.27 | 27.95 | | 24.48 | 31.42 |
| $C_2$ | 19.78 | 31.12 | $D_2$ | 22.05 | 27.04 | $H_2$ | 21.17 | 28.2 | $L_2$ | 23.07 | 29.68 |
| | 19.48 | 30.65 | | 22.13 | 27.01 | | 20.29 | 28.07 | | 22.77 | 29.53 |
| | 19.74 | 30.63 | | 22.16 | 27.06 | | 20.14 | 28.12 | | 22.35 | 29.55 |
| $C_3$ | 19.29 | 30.77 | $D_3$ | 22.95 | 27.87 | $H_3$ | 19.23 | 26.51 | $L_3$ | 23.00 | 29.73 |
| | 19.63 | 30.86 | | 22.84 | 28.1 | | 19.08 | 26.77 | | 23.19 | 29.82 |
| | 19.66 | 30.58 | | 23.89 | 28.6 | | 19.32 | 26.97 | | 22.87 | 29.91 |
| $C_4$ | 19.54 | 30.62 | $D_4$ | 23.09 | 28.01 | $H_4$ | 18.97 | 26.40 | $L_4$ | 18.38 | 25.26 |
| | 19.94 | 30.89 | | 23.26 | 28.01 | | 18.44 | 26.37 | | 18.52 | 25.59 |
| | 19.89 | 31.6 | | 23.55 | 28.87 | | 19.27 | 26.59 | | 18.62 | 25.19 |

## DATA PROCESSING

The mean value of the number of the Ct values corresponding to every inflammatory indicator will be calculated for four categories: the control, DSS-intervenue, high, and low groups.

$$Average(\overline{X}) = \frac{Total\ value\ of\ all\ trials}{Number\ of\ trials}$$

Taking the data of the Interleukin-10 group as an example:

*Table 5: Calculation of mean Ct values*

| Ct | $C_1$ | | | $C_2$ | | | $C_3$ | | | $C_4$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IL-10 | 32.69 | 32.05 | 32.02 | 32.79 | 32.95 | 33.8 | 32.74 | 32.52 | 32.79 | 31.59 | 31.73 | 31.41 |
| $Mean_1$ | 32.25 | | | 33.18 | | | 32.68 | | | 31.58 | | |
| GAP | 18.33 | 18.12 | 18.09 | 19.55 | 19.25 | 19.51 | 18.61 | 19.02 | 18.87 | 17.23 | 17.44 | 17.44 |
| $Mean_2$ | 18.18 | | | 19.44 | | | 18.83 | | | 17.37 | | |
| $M_1$-$M_2$ | 32.25 - 18.18 = 14.07 | | | 33.18 - 19.44 = 13.74 | | | 32.68 - 18.83 = 13.85 | | | 31.58 - 17.37 = 14.21 | | |
| $Mean_3$ | Average (C) = $\frac{14.07 + 13.74 + 13.85 + 14.21}{4}$ = 13.97 | | | | | | | | | | | |

Standard deviation was also calculated for the Ct values corresponding to every indicator, and the value of target genes was compared with GAPDH. Taking the data for the Ct values of GAPDH in the IL-10 control group as an example:

*Table 6: Calculation of standard deviation of Ct values using data collected from the IL-10 control group*

| GAP | Average($\overline{X}$) | $(X - \overline{X})$ | $(X - \overline{X})^2$ | $\sigma = \sqrt{\frac{(X-\overline{X})^2}{n-1}}$ |
|---|---|---|---|---|
| 18.33 | $\overline{X} = \frac{18.33+18.12+18.09}{3}$ = 18.18 | -0.15 | 0.023 | $\sigma = \sqrt{\frac{0.023+0.004+0.008}{3-1}}$ = $\sqrt{0.0175}$ = 0.132 |
| 18.12 | | 0.06 | 0.004 | |
| 18.09 | | 0.09 | 0.008 | |

After obtaining the Ct mean values of the internal reference gene GAPDH and the inflammatory factor gene, the average subtraction values between the two sets of data was calculated using biological replicates.

Taking the Ct of GAPDH and IL-10 as an example:

$$\overline{\Delta\overline{Ct}} = \frac{\Delta\overline{Ct}_{C1} + \Delta\overline{Ct}_{C2} + \Delta\overline{Ct}_{C3}}{number\ of\ biological\ replicates}$$

The relative quantification was also calculated:

$$RQ = 2^{-\overline{\Delta\Delta Ct}}$$

The purpose of relative quantification is to determine the relative proportion of the content of the target gene in two or more samples, without the need to know their copy number in each sample.

## DATA ANALYSIS

### GRAPHICAL ANALYSIS

The relative quantification obtained through the calculation of the Ct values of the target gene and the housekeeping gene is presented in a bar chart in figures 4-7, showing the general trend of the data. Relative quantification is used to determine the difference in the amount of target genes in two or more different samples, and the obtained data is the relative proportion of the content of target genes in each sample.

The Ct values of target genes in the experimental sample will be compared with those in the control sample. The data obtained from relative quantification reflect the fold change between the starting amount of the target gene and the amount detected after amplification. It is usually used in the data analysis of fluorescence real-time quantitative PCR to determine the changes of the target gene before and after treatment.

In this study, a single factor analysis of variance (ANOVA) test was conducted to collect statistical models between the average values under the influence of a specific independent variable at different levels. If the difference between the control group, DSS induced inflammation group and high-dose group among the four genes is statistically significant in the experimental group, it can fully prove the alternative hypothesis. A p value less than 0.05 indicates statistical significance, suggesting that the likelihood of the observed differences occuring by chance is very low, and thus the null hypothesis can be rejected.

A. **Relative Quantitation of target gene IL-1β**

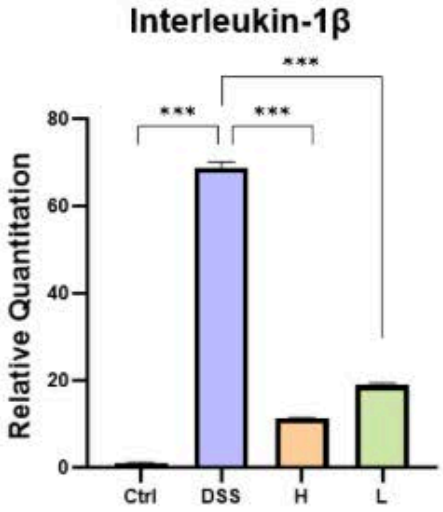



Figure 4: Relative quantitation of target gene IL-1β. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

B. **Relative Quantitation of target gene IL-6**

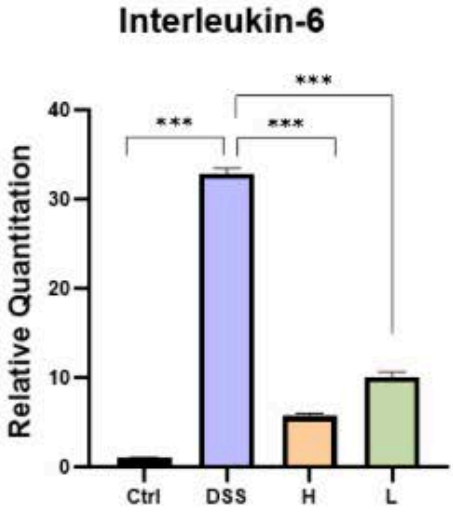



Figure 5: Relative quantitation of target gene IL-6. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

C. **Relative Quantitation of target gene IL-10**

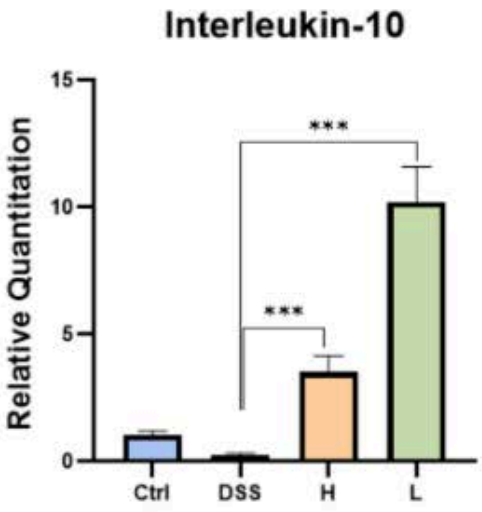



Figure 6: Relative quantitation of target gene IL-10. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

D. **Relative Quantitation of target gene Claudin-1**

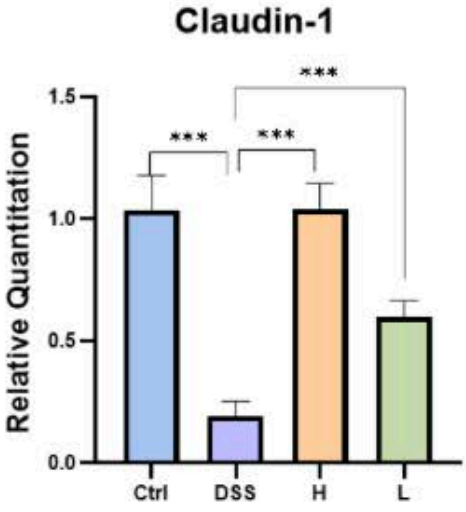



Figure 7: Relative quantitation of target gene Claudin-1. The values are expressed as means + SE of three parallel trials (n=3). The number of asterisks (*) next to the bars represents level of significant difference ***P ≤ 0.00l by single-factor ANOVA analysis using PRISM (8.0.2)

## RESULT ANALYSIS

Both alternative hypotheses have been fully demonstrated in the experiment. There will be a significant difference in the expression level of inflammatory factor genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) between colon cells receiving high and low concentrations of LV149 probiotic solutions.

### INTERLEUKIN-1β

It is observed from the graph that the DSS group has the greatest value of relative quantification: up to 68.87. The high-dose group leads to an decrement of relative quantification level to 11.35, and the low-dose group exhibits a relative quantification level of 18.96. Data collected fully proves the expression of the proinflammatory factor gene in the four experimental groups.

### INTERLEUKIN-6

As a pro-inflammatory and anti-inflammatory factor similar to IL-1β, IL-6 showed a significant increase in expression in the DSS treatment group. In the treatment of high-dose *B. pumilus* solution, IL-6 showed a relatively lower expression level, reaching a relative quantification of 5.72, and a relative quantification of 10.09 under low-dose treatment with probiotics. The results reflect the therapeutic effect of IL-6 on probiotics, especially in high concentration solutions with sensitive reactions.

### INTERLEUKIN-10

IL-10 is an anti-inflammatory factor gene. In the DSS-induced group, the relative expression of the IL-10 gene decreased to 0.274 compared to the control group. In the high-dose group, the IL-10 gene showed a relative quantitative value of 3.52, which was far less than the relative quantitative value of 10.19 in the low-dose probiotic group.

### CLAUDIN-1

Claudin-1 is an indicator reflecting the permeability of intestinal epithelial cell barrier. According to the chart, the DSS induced inflammation group showed a lower expression compared to the control group, reaching a relative quantification value of 0.192. In the high and low dose groups, the relative quantitative values were 1.04 and 0.59, respectively.

## DISCUSSION

In the preliminary experiment, using an interleukin gene as the target, it was ultimately determined that the expression level of the inflammation related gene was significantly increased in the bacterial solution at two concentrations of $1\times10^8$ CFU/mL and 1×109 CFU/mL within the range of $1\times10^5$, $1\times10^6$, $1\times10^7$, $1\times10^8$, $1\times10^9$ and $1\times10^{10}$ CFU/mL. Therefore, within the scope of this article's exploration, the changes in the expression levels of three interleukin genes (IL-1β, IL-6 and IL-10) and a tight junction protein gene (Claudin-1) were investigated using the concentrations of these two bacterial solutions as variables.

### AN EXPLANATION OF THE POSITIVE CORRELATION BETWEEN LV-149 AND THE ALLEVIATION OF UC

Further evidence suggests that the colonization of probiotics in the intestine helps to form an antibacterial environment and reduce the production of pro-inflammatory cytokines. There are reports that *B. pumilus* and its metabolites can activate the NF- κB signal NLRP3 inflammasome and inhibit IL-1β secretion (Ahn et al., 2011). This study has also yielded similar results, with supplementation of *B. pumilus* reducing IL-1β, IL-6, TNF-α and increasing the level of IL-10. One possible explanation for these findings is that activated PPAR-γ mediates the inhibition of pro-inflammatory cytokines such as TNF-α and IL-1β. Additionally, the production of IL-6 may contribute to anti-inflammatory effects. TGF-β plays a role in reducing intestinal mucosal damage by inhibiting the production of inflammatory factors from macrophages (Maheshwari et al., 2011). In this experiment, the addition of *Bacillus pumilus* increased IFN-γ levels. Although IFN-γ is an effective immune molecule against pathogenic microbes, its abnormal elevation is associated with autoimmune dysregulation and alterations in the gut microbiota. Excessive IFN-γ activation can also lead to tissue damage, necrosis, and inflammation (Sun & Guo et al., 2022). The intestinal mucosal layer is secreted by goblet cells distributed throughout the gastrointestinal tract. In this study, administration of *B. pumilus* appeared to increase the thickness of the muscular layer, possibly due to improvements

in gut microbiota composition. Notably, the expression of Claudin-1, a key tight junction protein, was elevated in the experimental group. This aligns with findings by Sheng et al., who reported that *B. pumilus* reduced epithelial barrier damage by upregulating tight junction protein expression. The observed changes in Claudin-1 expression in our study were consistent across the three treatment groups.

## CONCLUSION

The analysis in this study considers the characteristics of each gene. IL-6 and IL-1 β are proinflammatory factor genes–that is, they are largely expressed in inflammatory conditions. The DSS group in the chart often has a higher relative quantitative value compared with the control group and the high-dose group, which also proves the correctness of this theory from the experimental level. IL-10 is an anti-inflammatory factor gene, and has a relatively sensitive response to low-dose probiotics–that is, the best concentration of efficacy. Claudin-1, to a certain extent, acts as a response to the permeability of intestinal epithelial cells–that is, its expression level is reduced in the DSS induced group due to the thinning of the intestinal barrier caused by inflammation. In the following high and low dose groups, there was a significant increase in the expression level of the Claudin-1 gene, with the high-dose group showing the most significant increase. This shows that the concentration of probiotic solution for the best therapeutic effect belongs to the high-dose group, which has the important function of improving intestinal mucosal permeability.

## FUTURE DIRECTIONS

This experiment successfully evaluated the alleviating effect of *B. pumilus* LV149 on UC mice and the repairing effect of this bacteria on colon mucosal damage. Additionally, the study effectively provided theoretical guidance for the future relief and treatment of ulcerative colitis with live probiotics. While probiotics such as *Bacillus pumilus* have been explored for their potential in treating human diseases, there are currently no published studies specifically investigating the role of *B. pumilus* in alleviating clinical symptoms of UC or in repairing colonic mucosal damage

At present, the screening of probiotics is mainly determined based on their tolerance to gastrointestinal conditions (gastric acid and bile), ability to adhere to the gastrointestinal mucosa, and competitive rejection ability against pathogens. However, for probiotics to have a positive effect in the host's body, they must have a significant beneficial effect on the host, be non toxic, be non pathogenic, and have no obvious adverse effects. They also must be able to survive in the gastrointestinal tract with a sufficient number of cells and maintain probiotic properties under storage and processing conditions. Numerous studies have reported the potential clinical efficacy of probiotics and their formulations in the treatment of many diseases, such as functional dyspepsia.

Although modern molecular technology has become popular, the mechanisms involved are explored through changes in certain protein levels to explore possible pathways. Further studies may delve into the transcriptome gene differences and explore the role of *B. pumilus* in alleviating UC, which is a relatively novel and accurate biological method. Under the conditions of this experiment, *B. pumilus* can improve the morphology of colon tissue, regulate the content of inflammatory cytokines and related serum indicators, increase the content of colonic tight junction protein, and reduce tissue inflammatory cell infiltration.

## REFERENCES

1. Ahn, Huijeong, et al. “Poly-Gamma-Glutamic Acid from Bacillus Subtilis Upregulates Pro-Inflammatory Cytokines While Inhibiting NLRP3, NLRC4 and AIM2 Inflammasome Activation.” *Cellular & Molecular Immunology*.

2. Cayman Chemical. *Disuccinimidyl Suberate (DSS)*.

3. Chen, Yun, et al. “A Role of Exopolysaccharide Produced by Streptococcus Thermophilus in the Intestinal Inflammation and Mucosal Barrier in Caco-2 Monolayer and Dextran Sulphate Sodium-Induced Experimental Murine Colitis.” *Molecules*, vol. 24, no. 3, 31 Jan. 2019, p. 513

4. Dai, Yun, et al. “Medium Optimization to Analyze the Protein Composition of Bacillus Pumilus HR10 Antagonizing Sphaeropsis.” *AMB Express.*

5. D'Amelio, P., and F. Sassi. "Gut Microbiota, Immune System, and Bone." *Calcified Tissue International*, vol. 102, no. 4, 30 Sept. 2017, pp. 415–425.

6. DeCaprio, James, and Thomas O. Kohl. "Cross-Linking Antibodies to Beads with Disuccinimidyl Suberate (DSS)." *Cold Spring Harbor Protocols*, vol. 2019, no. 2, Feb. 2019, p. pdb.prot098632.

7. Fahey, James K., et al. "The Intercellular Tight Junction and Spontaneous Coronary Artery Dissection." *Journal of the American College of Cardiology*, vol. 72, no. 14, 1 Oct. 2018, pp. 1752–1753.

8. Friedrich, Matthias, et al. "Cytokine Networks in the Pathophysiology of Inflammatory Bowel Disease." *Immunity*, vol. 50, no. 4, Apr. 2019, pp. 992–1006.

9. Hunter, Christopher A., and Simon A. Jones. "IL-6 as a Keystone Cytokine in Health and Disease." *Nature Immunology*, vol. 16, no. 5, 21 Apr. 2015, pp. 448–457.

10. Johansson, Malin E. V. "Fast Renewal of the Distal Colonic Mucus Layers by the Surface Goblet Cells as Measured by in Vivo Labeling of Mucin Glycoproteins." *PLoS ONE*, vol. 7, no. 7, 17 July 2012, p. e41009.

11. KITAJIMA, Shuji, et al. "Changes in Colonic Mucosal Permeability in Mouse Colitis Induced with Dextran Sulfate Sodium." *Experimental Animals*, vol. 48, no. 3, 1999, pp. 137–143.

12. Luo, Peng, et al. *Preparation of Bacillus Short Bacteria and Probiotics and Its Preparation Method and Application*. 19 June 2013.

13. Maheshwari, Akhil, et al. "TGF-β2 Suppresses Macrophage Cytokine Production and Mucosal Inflammatory Responses in the Developing Intestine." *Gastroenterology*, vol. 140, no. 1, 1 Jan. 2011.

14. Merga, Yvette, et al. "Mucosal Barrier, Bacteria and Inflammatory Bowel Disease: Possibilities for Therapy." *Digestive Diseases*, vol. 32, no. 4, 2014, pp. 475–483,

15. Saggese, Anella, et al. "An Antimicrobial Peptide Specifically Active against Listeria Monocytogenes Is Secreted by Bacillus Pumilus SF214." *BMC Microbiology*, vol. 22, no. 1, 3 Jan. 2022.

16. Shao, Ying, et al. "The Role of Intestinal Microbiota in Inflammatory Bowel Disease." *Antibiotics*, vol. 10, no. 6, 2021, p. 643.

17. Sheng, Kangliang, et al. "Probiotic Bacillus Cereus Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice through Improvement of the Intestinal Barrier Function, Anti-Inflammation, and Gut Microbiota Modulation." *Journal of Agricultural and Food Chemistry*, vol. 69, no. 49, 22 Oct. 2021, pp. 14810–14823.

18. Sun, Lei, et al. "Quercetin Reduces Inflammation and Protects Gut Microbiota in Broilers." *Molecules*, vol. 27, no. 10, 19 May 2022, p. 3269.

19. Tan, Yue, et al. "Correlation of Intestinal Mucosal Healing and Tight Junction Protein Expression in Ulcerative Colitis Patients." *The American Journal of the Medical Sciences*, vol. 357, no. 3, 1 Mar. 2019, pp. 195–204.

20. Vancamelbeke, Maaike, et al. "Genetic and Transcriptomic Basis of Intestinal Epithelial Barrier Dysfunction in Inflammatory Bowel Disease." *Inflammatory Bowel Diseases*, vol. 23, no. 10, 1 Oct. 2017, pp. 1718–1729.

21. Vindigni, Stephen M., et al. "The Intestinal Microbiome, Barrier Function, and Immune System in Inflammatory Bowel

22. Zeng, Zhibo, et al. "Probiotic Potential of Bacillus Licheniformis and Bacillus Pumilus Isolated from Tibetan Yaks, China." *Probiotics and Antimicrobial Proteins*, 20 Apr. 2022, pp. 579–594.

*Table 7: Calculation of Standard deviations of Ct values of IL-1β, IL-6, IL-10 and Claudin*

| Standard Deviation of Ct values | | | | |
|---|---|---|---|---|
| | **IL-1β** | **IL-6** | **IL-10** | **Claudin-1** |
| $C_{1\ STD}$ | 0.106 | 0.038 | 0.068 | 0.117 |
| $C_{2\ STD}$ | 0.062 | 0.095 | 0.143 | 0.204 |
| $C_{3\ STD}$ | 0.076 | 0.031 | 0.087 | 0.041 |
| $C_{4\ STD}$ | 0.111 | 0.077 | 0.057 | 0.178 |
| $D_{1\ STD}$ | 0.079 | 0.060 | 0.106 | 0.071 |
| $D_{2\ STD}$ | 0.037 | 0.019 | 0.055 | 0.125 |
| $D_{3\ STD}$ | 0.029 | 0.063 | 0.024 | 0.127 |
| $D_{4\ STD}$ | 0.079 | 0.136 | 0.039 | 0.246 |
| $H_{1\ STD}$ | 0.137 | 0.056 | 0.107 | 0.062 |
| $H_{2\ STD}$ | 0.049 | 0.050 | 0.115 | 0.099 |
| $H_{3\ STD}$ | 0.062 | 0.064 | 0.057 | 0.031 |
| $H_{4\ STD}$ | 0.015 | 0.107 | 0.094 | 0.0391 |
| $L_{1\ STD}$ | 0.137 | 0.054 | 0.058 | 0.034 |
| $L_{2\ STD}$ | 0.056 | 0.086 | 0.226 | 0.163 |
| $L_{3\ STD}$ | 0.068 | 0.023 | 0.027 | 0.132 |
| $L_{4\ STD}$ | 0.055 | 0.138 | 0.104 | 0.128 |

*Table 8: IL-1β data Ct processing table*

| **IL-1β Data Ct processing table** | | | | |
|---|---|---|---|---|
| ***Control*** | **$C_{M1}$** | **$C_{M2}$** | **$C_{M3}$** | **$C_{M4}$** |
| **GAP** | 18.72 | 19.67 | 19.53 | 18.79 |
| **IL-1β** | 29.32 | 30.8 | 30.74 | 31.037 |
| **D-value** | 10.60 | 11.13 | 11.21 | 12.25 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (C)** = $\frac{10.60 + 11.13 + 11.21 + 12.25}{4}$ = 11.29 | | | |
| ***DSS*** | **$D_{M1}$** | **$D_{M2}$** | **$D_{M3}$** | **$D_{M4}$** |
| **GAP** | 20.3 | 22.81 | 23.36 | 21.3 |
| **IL-1β** | 29.24 | 27.19 | 28.19 | 28.29 |
| **D-value** | 8.94 | 4.37 | 4.83 | 6.99 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (D)** = $\frac{8.94 + 4.37 + 4.83 + 6.99}{4}$ = 6.29 | | | |
| ***High*** | **$H_{M1}$** | **$H_{M2}$** | **$H_{M3}$** | **$H_{M4}$** |
| **GAP** | 21.69 | 21.57 | 19.21 | 18.46 |
| **IL-1β** | 27.79 | 27.03 | 24.75 | 26.45 |
| **D-value** | 6.09 | 5.47 | 5.54 | 7.99 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (H)** = $\frac{6.09 + 5.47 + 5.54 + 7.99}{4}$ = 6.27 | | | |
| ***Low*** | **$L_{M1}$** | **$L_{M2}$** | **$L_{M3}$** | **$L_{M4}$** |
| **GAP** | 24.31 | 22.16 | 23.25 | 18.51 |
| **IL-1β** | 31.13 | 27.59 | 28.62 | 25.61 |
| **D-value** | 6.82 | 5.42 | 5.37 | 7.11 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (L)** = $\frac{6.82 + 5.42 + 5.37 + 7.11}{4}$ = 6.18 | | | |

*Table 9: Relative quantification value of four genes calculated using the logarithm method.*

| Relative quantification of qPCR data | | | | |
|---|---|---|---|---|
| | $C_{D\text{-value}}$ | $D_{D\text{-value}}$ | $H_{D\text{-value}}$ | $L_{D\text{-value}}$ |
| **IL-1β** | 1 | -5.013 | -5.026 | -5.118 |
| **RQ** ($2^{-\Delta\Delta\overline{\overline{Ct}}}$) | | 32.278 | 32.578 | 34.735 |
| **IL-6** | 1 | -4.894 | -7.941 | -5.683 |
| **RQ** ($2^{-\Delta\Delta\overline{\overline{Ct}}}$) | | 29.737 | 245.714 | 51.357 |
| **IL-10** | 1 | -2.904 | -2.690 | -3.097 |
| **RQ** ($2^{-\Delta\Delta\overline{\overline{Ct}}}$) | | 7.486 | 6.453 | 8.554 |
| **Claudin-1** | 1 | 0.752 | 0.510 | 0.278 |
| **RQ** ($2^{-\Delta\Delta\overline{\overline{Ct}}}$) | | 0.594 | 0.702 | 0.825 |

*Table 10: Ct value of Interleukin-6 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution*

| **Interleukin-6 RT-qPCR Ct Value Data** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| group | GAP | IL-6 | group | GAP | IL-6 | group | GAP | IL-6 | group | GAP | IL-6 |
| $C_1$ | 20.37 | 35.32 | $D_1$ | 21.44 | 30.79 | $H_1$ | 21.01 | 33.22 | $L_1$ | 21.72 | 33.12 |
| | 20.28 | 35.19 | | 20.95 | 31.19 | | 20.85 | 33.38 | | 21.59 | 33.04 |
| | 20.34 | 35.51 | | 20.7 | 31.14 | | 20.82 | 33.44 | | 21.51 | 33.27 |
| $C_2$ | 20.01 | 35.01 | $D_2$ | 21.58 | 31.53 | $H_2$ | 20.21 | 32.19 | $L_2$ | 21.56 | 33.49 |
| | 20.07 | 35.26 | | 21.63 | 31.64 | | 20.04 | 32.88 | | 21.43 | 33.24 |
| | 20.19 | 35.25 | | 21.66 | 31.53 | | 20.07 | 32.56 | | 21.95 | 33.31 |
| $C_3$ | 20.38 | 35.92 | $D_3$ | 21.1 | 31.44 | $H_3$ | 20.11 | 32.42 | $L_3$ | 21.68 | 33.13 |
| | 20.21 | 34.94 | | 21.32 | 31.43 | | 20.01 | 32.34 | | 21.68 | 33.31 |
| | 20.28 | 35.02 | | 21.14 | 30.53 | | 19.82 | 32.4 | | 21.56 | 33.35 |
| $C_4$ | 20.34 | 34.97 | $D_4$ | 20.97 | 30.54 | $H_4$ | 18.82 | 31.69 | $L_4$ | 20.32 | 31.59 |
| | 20.57 | 35.14 | | 21.12 | 30.91 | | 19.31 | 31.46 | | 19.59 | 31.32 |
| | 20.25 | 35.34 | | 20.07 | 30.8 | | 18.97 | 31.75 | | 19.37 | 31.65 |

**APPENDIX - 5**

*Table 11: Ct value of Interleukin-10 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution*

| Interleukin-10 RT-qPCR Ct Value Data | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| group | GAP | IL-10 | group | GAP | IL-10 | group | GAP | IL-10 | group | GAP | IL-10 |
| $C_1$ | 18.33 | 32.69 | $D_1$ | 18.14 | 34.65 | $H_1$ | 21.47 | 33.98 | $L_1$ | 19.73 | 30.08 |
| | 18.12 | 32.05 | | 18.08 | 34.19 | | 21.47 | 33.71 | | 19.8 | 30.25 |
| | 18.09 | 32.02 | | 18.53 | 34.26 | | 21.65 | 33.78 | | 19.43 | 30.19 |
| $C_2$ | 19.55 | 32.79 | $D_2$ | 18.32 | 34.41 | $H_2$ | 22.18 | 34.31 | $L_2$ | 22.09 | 31.91 |
| | 19.25 | 32.95 | | 18.58 | 34.32 | | 22.59 | 34.04 | | 22.25 | 33.73 |
| | 19.51 | 33.8 | | 18.42 | 34.51 | | 22.61 | 34.97 | | 22.55 | 33.1 |
| $C_3$ | 18.61 | 32.74 | $D_3$ | 18.98 | 34.52 | $H_3$ | 22.95 | 35.14 | $L_3$ | 22.34 | 33.28 |
| | 19.02 | 32.52 | | 18.96 | 34.46 | | 23.18 | 35.19 | | 22.24 | 33.26 |
| | 18.87 | 32.79 | | 18.23 | 34.6 | | 23.05 | 34.82 | | 22.54 | 33.36 |
| $C_4$ | 17.23 | 31.59 | $D_4$ | 18.18 | 33.71 | $H_4$ | 22.43 | 35.3 | $L_4$ | 23.17 | 33.54 |
| | 17.44 | 31.73 | | 18.08 | 33.85 | | 23 | 35.18 | | 23.2 | 34.03 |
| | 17.44 | 31.41 | | 18.22 | 33.71 | | 22.95 | 35.45 | | 23.01 | 33.71 |

*Table 12: Ct value of Claudin-1 in mice colon cells collected from qPCR instrument under the treatment of DSS solution, high, and low dose of LV-149 probiotic strain solution*

| **Claudin-1 RT-qPCR Ct Value Data** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| group | GAP | Claudin-1 | group | GAP | Claudin-1 | group | GAP | Claudin-1 | group | GAP | Claudin-1 |
| $C_1$ | 19.35 | 29.91 | $D_1$ | 20.49 | 33.07 | $H_1$ | 23.92 | 33.76 | $L_1$ | 20.18 | 31.35 |
| | 19.92 | 29.94 | | 20.49 | 33.05 | | 23.94 | 34.06 | | 19.96 | 31.28 |
| | 19.41 | 29.39 | | 20.01 | 33.13 | | 23.79 | 34.08 | | 20.37 | 31.44 |
| $C_2$ | 19.18 | 28.48 | $D_2$ | 20.59 | 33.05 | $H_2$ | 22.98 | 32.88 | $L_2$ | 22.89 | 33.69 |
| | 19.23 | 29.48 | | 20.53 | 33.21 | | 22.97 | 33.59 | | 22.73 | 33.62 |
| | 19.18 | 29.96 | | 20.38 | 33.72 | | 22.82 | 33.45 | | 22.95 | 33.89 |
| $C_3$ | 19.87 | 30.2 | $D_3$ | 20.24 | 33.38 | $H_3$ | 23.69 | 33.85 | $L_3$ | 22.77 | 33.29 |
| | 19.89 | 30.24 | | 20.43 | 33.15 | | 23.38 | 33.82 | | 22.74 | 33.53 |
| | 19.78 | 30.38 | | 20.1 | 33.9 | | 23.43 | 33.82 | | 23.11 | 34.57 |
| $C_4$ | 19.23 | 29.77 | $D_4$ | 22.54 | 34.46 | $H_4$ | 23.21 | 33.37 | $L_4$ | 22.53 | 33.53 |
| | 19.37 | 29.55 | | 22.26 | 34.4 | | 23.36 | 33.3 | | 22.39 | 33.21 |
| | 19.26 | 29.53 | | 22.41 | 34.73 | | 23.16 | 33.37 | | 22.65 | 34.36 |

*Table 13: IL-6 Ct data processing table*

| **IL-6 Data Ct processing table** | | | | |
|---|---|---|---|---|
| ***Control*** | **$C_{M1}$** | **$C_{M2}$** | **$C_{M3}$** | **$C_{M4}$** |
| **GAP** | 20.33 | 20.09 | 20.29 | 20.39 |
| **IL-6** | 34.34 | 35.41 | 35.96 | 35.15 |
| **D-value** | 14.01 | 15.32 | 15.67 | 14.76 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (C)** = $\frac{14.01 + 15.32 + 15.67 + 14.76}{4}$ = 14.94 | | | |
| ***DSS*** | **$D_{M1}$** | **$D_{M2}$** | **$D_{M3}$** | **$D_{M4}$** |
| **GAP** | 21.03 | 21.62 | 21.19 | 20.72 |
| **IL-6** | 31.53 | 31.57 | 30.77 | 30.88 |
| **D-value** | 10.49 | 9.94 | 9.58 | 10.16 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (D)** = $\frac{10.49 + 9.94 + 9.58 + 10.16}{4}$ = 10.05 | | | |
| ***High*** | **$H_{M1}$** | **$H_{M2}$** | **$H_{M3}$** | **$H_{M4}$** |
| **GAP** | 23.89 | 22.07 | 20.05 | 19.0 |
| **IL-6** | 30.81 | 26.21 | 26.39 | 29.63 |
| **D-value** | 6.92 | 4.14 | 6.34 | 10.6 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (H)** = $\frac{6.92 + 4.14 + 6.34 + 10.6}{4}$ = 6.99 | | | |
| ***Low*** | **$L_{M1}$** | **$L_{M2}$** | **$L_{M3}$** | **$L_{M4}$** |
| **GAP** | 21.61 | 21.65 | 21.64 | 18.76 |
| **IL-6** | 33.57 | 30.21 | 29.36 | 27.52 |
| **D-value** | 11.98 | 8.57 | 7.72 | 8.76 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (L)** = $\frac{11.98 + 8.57 + 7.72 + 8.76}{4}$ = 9.26 | | | |

Text

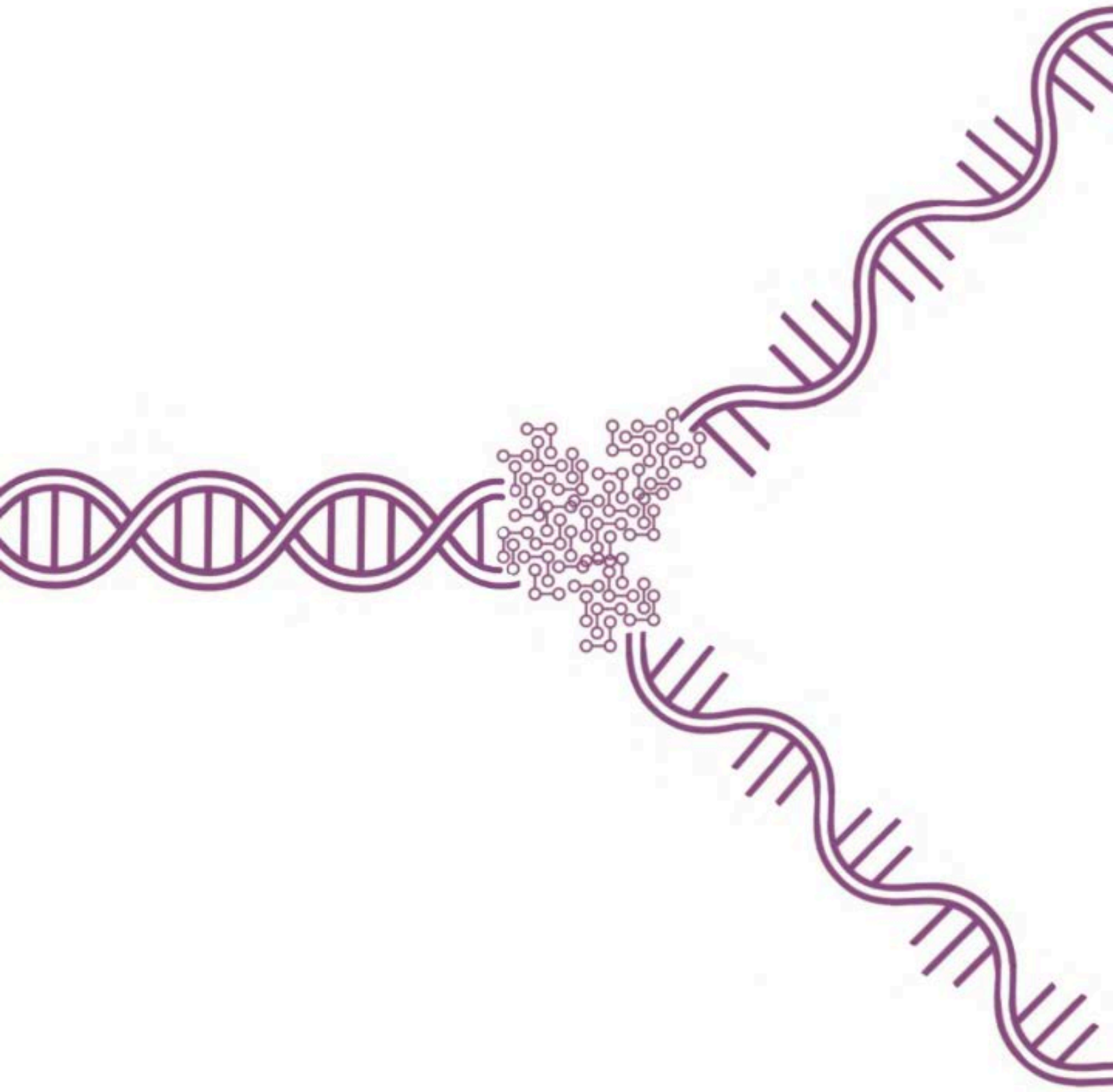
*Table 14: IL-10 Ct data processing table*

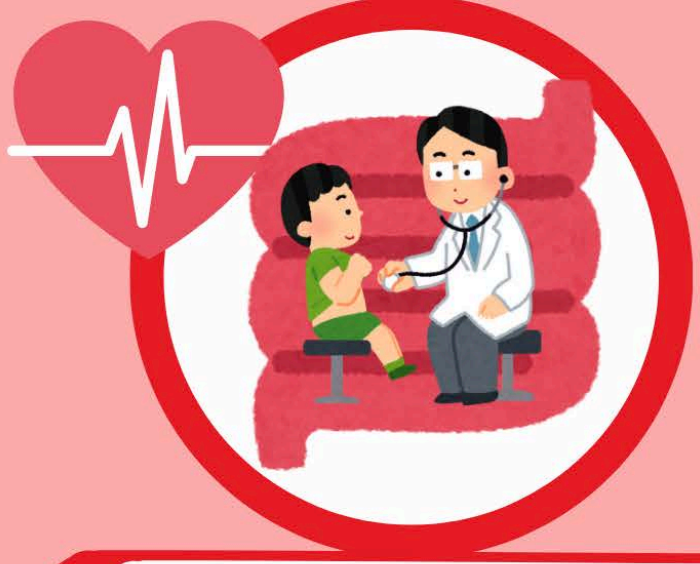
| **IL-10 Data Ct processing table** | | | | |
|---|---|---|---|---|
| ***Control*** | $C_{M1}$ | $C_{M2}$ | $C_{M3}$ | $C_{M4}$ |
| **GAP** | 18.18 | 19.44 | 18.83 | 17.37 |
| **IL-10** | 32.25 | 33.18 | 32.68 | 31.58 |
| **D-value** | 14.07 | 13.74 | 13.85 | 14.21 |
| **Mean** $_{\text{D-value}}$ | $\text{Average}_{Ct}\,(C) = \frac{14.07 + 13.74 + 13.85 + 14.21}{4} = 13.97$ | | | |
| ***DSS*** | $D_{M1}$ | $D_{M2}$ | $D_{M3}$ | $D_{M4}$ |
| **GAP** | 20.25 | 22.44 | 21.06 | 22.06 |
| **IL-10** | 32.37 | 32.41 | 31.53 | 33.76 |
| **D-value** | 12.12 | 9.97 | 10.47 | 11.69 |
| **Mean** $_{\text{D-value}}$ | $\text{Average}_{Ct}\,(D) = \frac{12.12 + 9.97 + 10.47 + 11.69}{4} = 11.06$ | | | |
| ***High*** | $H_{M1}$ | $H_{M2}$ | $H_{M3}$ | $H_{M4}$ |
| **GAP** | 23.53 | 22.46 | 23.06 | 22.79 |
| **IL-10** | 32.49 | 33.11 | 35.05 | 36.31 |
| **D-value** | 8.96 | 10.65 | 11.99 | 13.52 |
| **Mean** $_{\text{D-value}}$ | $\text{Average}_{Ct}\,(H) = \frac{8.96 + 10.65 + 11.99 + 13.52}{4} = 11.28$ | | | |
| ***Low*** | $L_{M1}$ | $L_{M2}$ | $L_{M3}$ | $L_{M4}$ |
| **GAP** | 19.65 | 22.29 | 22.37 | 22.34 |
| **IL-10** | 30.17 | 32.91 | 33.3 | 33.76 |
| **D-value** | 10.52 | 10.62 | 10.93 | 11.42 |
| **Mean** $_{\text{D-value}}$ | $\text{Average}_{Ct}\,(L) = \frac{10.52 + 10.62 + 10.93 + 11.42}{4} = 10.87$ | | | |

*Table 15: Claudin-1 data Ct processing table*

| **Claudin-1 Ct Data processing table** | | | | |
|---|---|---|---|---|
| ***Control*** | **$C_{M1}$** | **$C_{M2}$** | **$C_{M3}$** | **$C_{M4}$** |
| **GAP** | 19.56 | 19.19 | 19.85 | 19.29 |
| **Claudin-1** | 28.11 | 29.31 | 30.27 | 31.28 |
| **D-value** | 8.55 | 10.11 | 10.43 | 11.99 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (C)** = $\frac{8.55 + 10.11 + 10.43 + 11.99}{4}$ = 10.27 | | | |
| ***DSS*** | **$D_{M1}$** | **$D_{M2}$** | **$D_{M3}$** | **$D_{M4}$** |
| **GAP** | 20.33 | 20.5 | 20.26 | 22.40 |
| **Claudin-1** | 31.75 | 33.33 | 29.98 | 32.53 |
| **D-value** | 11.42 | 12.83 | 9.72 | 10.13 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (D)** = $\frac{11.42 + 12.83 + 9.72 + 10.13}{4}$ = 11.02 | | | |
| ***High*** | **$H_{M1}$** | **$H_{M2}$** | **$H_{M3}$** | **$H_{M4}$** |
| **GAP** | 23.88 | 22.92 | 23.5 | 23.24 |
| **Claudin-1** | 33.97 | 33.17 | 35.19 | 34.35 |
| **D-value** | 10.08 | 10.25 | 11.69 | 11.10 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (H)** = $\frac{10.08 + 10.25 + 11.69 + 11.10}{4}$ = 10.78 | | | |
| ***Low*** | **$L_{M1}$** | **$L_{M2}$** | **$L_{M3}$** | **$L_{M4}$** |
| **GAP** | 20.17 | 22.86 | 22.87 | 22.52 |
| **Claudin-1** | 30.36 | 32.77 | 33.79 | 33.70 |
| **D-value** | 10.19 | 9.91 | 10.92 | 11.18 |
| **Mean $_{D\text{-value}}$** | **$Average_{Ct}$ (L)** = $\frac{10.19 + 9.91 + 10.92 + 11.18}{4}$ = 10.55 | | | |

# SHORTS





# Colorectal CANCER

## A Warning to the Young

Colorectal cancer is found in the colon or rectum. It often starts as benign polyps (tumors). With time, these benign polyps then turn dangerously cancerous if not removed by a colonoscopy or surgery (National Cancer Institute, n.d).

In 2020, young Americans ages 15-19 saw a 300% increase in diagnoses and ages 20-24 saw a 185% increase (Bendix, 2023).

The change in diagnoses may be due to an adoption of more western lifestyles with heavily processed foods and minimal exercise (Siegal et al., 2017).

Warning: Do Not Self Diagnose. Seek Professional Help if You Have a Listed Symptom

Alarming symptoms include but are not limited to: blood in stool, change in bowel habits, frequent abdominal cramps (Bendix, 2023).


Bendix, A. (2023, March 1). Colon cancer rates have been rising for decades among younger people, new study finds. NBC News. https://www.nbcnews.com/health/health-news/colon-cancer-rates-rising-decades-younger-people-study-finds-rcna151343

National Cancer Institute. (n.d.). Colorectal cancer—Patient version. National Institutes of Health. https://www.cancer.gov/types/colorectal

Siegel, R. L., Miller, K. D., Fedewa, S. A., Ahnen, D. J., Meester, R. G. S., Barzi, A., & Jemal, A. (2017). Colorectal cancer statistics, 2017. Gut , 66(4), 683–691. https://doi.org/10.1136/gutjnl-2017-314915



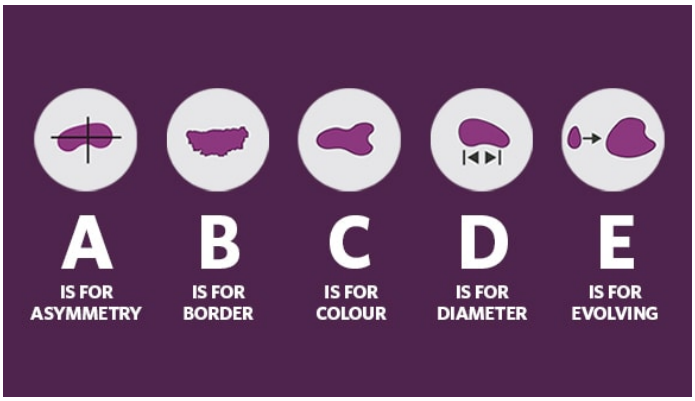

Andy Granados

# CANCER CAN BE SEEN: EARLY DETECTION OF SKIN CANCER CAN SAVE YOU

**BOLADE ABIOLA-FAGBA,** University of Illinois Urbana-Champaign

Surprisingly, the naked eye can identify skin cancer. A research article discovered that the common skin cancers are melanoma (malignant), which arises from cells that give the skin its color, and nonmelanoma skin cancers, which grow from other types of skin cells, mostly originating from UV rays or tanning beds (Marks, 1995). When skin cancer is detected in an individual, it often develops to its latest stage. The early detection of skin cancer can increase one's survival rate. An article published in the journal *Seminar in Oncology Nursing* reveals that melanoma self-detection rates range from 40% to 55% (Loescher et al., 2013). It has been proven that the early detection of skin cancer can increase one's survival rate as treatment starts earlier. It was highlighted in the article that the rate of melanoma mortality may decrease by 66% when detected early.

To decrease the mortality rate of skin cancer, it is essential to identify different ways to identify skin cancer early. Early detection through skin self-examinations and professional skin checks is crucial for improving outcomes, particularly for melanoma, where early-stage detection is associated with significantly higher survival rates (Carter, 2024). With all the information that has been presented, one might be questioning how skin cancer can be easily detected on one's skin. A guide created by the American Cancer Society, known as the ABCDE guideline, has been recognized as an effective way to detect skin cancer. Identifying early-stage skin cancers helps maintain a healthy lifestyle and can prevent life-threatening conditions and, in some cases, death. A simple physical check on your skin can save your life and benefit your health and lifestyle. Some health benefits of identifying skin cancer at an early stage are early treatment options, improved quality of life, reduced mortality rate, and reduced rate of treatments.




**REFERENCES**

1. Carter, E. (2025). *Identifying Types of Skin Cancer, Risk Factors, and Effective Treatments. International Journal of Advanced Engineering Technologies and Innovations*.

2. Henry, L., & Martin. (2025). *Prevent and Treat Skin Cancer. Advanced Dermatology & Skin Cancer Center*.

3. Loescher, L. J., Janda, M., Soyer, H. P., Shea, K., & Curiel-Lewandrowski, C. (2013). "Advances in Skin Cancer Early Detection and Diagnosis." *Seminars in Oncology Nursing*, vol. 29, no. 3, pp. 170–181.

4. Marks, R. (1995). *[Review of the article Wiley, by American Cancer Society]*.

5. Rigel, D. S., & Carucci, J. A. (2000). "Malignant Melanoma: Prevention, Early Detection, and Treatment in the 21st Century." *CA: A Cancer Journal for Clinicians*.

6. "Your Skin Check Checklist." *HCF Health Agenda*.

# WHAT TYPE OF CELL ARE YOU?

## WHAT'S YOUR SOCIAL STYLE?

A) I'M THE LIFE OF THE PARTY, CONSTANTLY CONNECTING WITH NEW PEOPLE.

B) I'M ALL ABOUT ACTION—IF THERE'S SOMETHING TO DO, I'M THE FIRST TO JUMP IN!

C) I'M A SOLO TRAVELER, ALWAYS ON THE MOVE AND HELPING OUT WHEREVER I'M NEEDED.

D) I'M THE PROTECTOR—I ALWAYS HAVE MY FRIENDS' BACKS AND HELP CLEAN UP MESSES.

E) I LIKE KEEPING THINGS NEAT AND ORGANIZED. YOU COULD SAY I'M THE GLUE THAT HOLDS THE GROUP TOGETHER.

F) I'M ADAPTABLE AND ALWAYS READY TO TAKE ON NEW ROLES. CHANGE IS MY MIDDLE NAME!

## WHICH OF THESE IS YOUR SUPERPOWER?

A) SUPER-FAST COMMUNICATION—I'M ALWAYS IN THE LOOP!

B) STRENGTH AND ENDURANCE—I'M READY FOR ANY CHALLENGE.

C) ADAPTABILITY—I CAN FIT IN ANYWHERE.

D) DEFENDING OTHERS—I'M NOT AFRAID TO FACE THREATS HEAD-ON.

E) CREATING A BARRIER—I KEEP EVERYTHING SAFE AND SOUND.

F) SHAPESHIFTING—I CAN TRANSFORM INTO WHATEVER IS NEEDED!

## HOW DO YOU DEAL WITH STRESS?

A) I THINK THINGS THROUGH LOGICALLY UNTIL I FIND A SOLUTION.

B) I BURN OFF STEAM THROUGH PHYSICAL ACTIVITY—NO TIME FOR SITTING STILL!

C) I TAKE DEEP BREATHS AND GO WITH THE FLOW.

D) I STEP UP AND FIGHT BACK AGAINST WHATEVER'S CAUSING THE PROBLEM.

E) I CREATE A CALM AND ORDERLY ENVIRONMENT TO KEEP THE STRESS AWAY.

F) I TRY OUT NEW WAYS TO HANDLE IT UNTIL I FIND WHAT WORKS.

## WHAT'S YOUR APPROACH TO CHALLENGES?

A) I THINK THINGS THROUGH AND MAKE SURE EVERYONE IS ON THE SAME WAVELENGTH.

B) I POWER THROUGH IT WITH DETERMINATION—NO OBSTACLE IS TOO TOUGH FOR ME.

C) I GO WITH THE FLOW AND MAKE SURE EVERYTHING STAYS IN BALANCE.

D) I TACKLE PROBLEMS HEAD-ON AND TAKE OUT ANY OBSTACLES IN MY PATH.

E) I BUILD BARRIERS AND KEEP EVERYTHING RUNNING SMOOTHLY.

F) I LIKE TO EXPLORE ALL MY OPTIONS BEFORE SETTLING ON THE BEST ONE.

## WHAT'S YOUR DREAM VACATION?

A) EXPLORING A NEW CITY AND LEARNING EVERYTHING ABOUT ITS HISTORY AND CULTURE—NEVER A DULL MOMENT!

B) A HIKING TRIP THROUGH RUGGED MOUNTAINS—WHO NEEDS RELAXATION WHEN YOU CAN CLIMB?

C) A ROAD TRIP, STOPPING AT EVERY QUIRKY SMALL TOWN ALONG THE WAY. I JUST KEEP MOVING!

D) VOLUNTEERING ABROAD, HELPING BUILD HOMES OR TEACH KIDS—MAKING A DIFFERENCE WHEREVER I GO.

E) A LUXURIOUS STAYCATION AT HOME, WHERE I CAN TIDY UP AND RECHARGE IN MY OWN SPACE.

F) AN ADVENTURE WHERE I CAN TRY SOMETHING NEW EVERY DAY—FROM SKYDIVING TO COOKING CLASSES!

## WHAT'S YOUR FAVORITE HOBBY?

A) PLAYING BRAIN GAMES OR SOLVING PUZZLES—IT KEEPS ME SHARP!

B) HITTING THE GYM OR GOING FOR A RUN. STAYING ACTIVE IS A MUST.

C) TRAVELING AND EXPLORING NEW PLACES. I'M ALWAYS ON THE MOVE!

D) VOLUNTEERING—I LOVE HELPING OUT AND MAKING A DIFFERENCE.

E) HOME IMPROVEMENT PROJECTS OR REDECORATING. I KEEP THINGS IN TIP-TOP SHAPE.

F) LEARNING NEW SKILLS. I LIKE TO KEEP MY OPTIONS OPEN.

## IF YOU GOT MOSTLY A'S.....

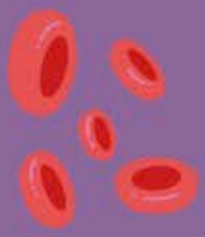
YOU'RE A NEURON! YOU'RE A NATURAL COMMUNICATOR, ALWAYS BUZZING WITH NEW IDEAS AND MAKING CONNECTIONS. LIKE A NEURON, YOU KEEP THINGS RUNNING SMOOTHLY BY KEEPING EVERYONE INFORMED!

## IF YOU GOT MOSTLY B'S.....

YOU'RE A MUSCLE CELL (MYOCYTE)! YOU'RE ALL ABOUT MOVEMENT AND ENERGY. WHETHER IT'S LIFTING, RUNNING, OR JUST BEING ACTIVE, YOU BRING THE POWER–JUST LIKE A MUSCLE CELL.

## IF YOU GOT MOSTLY C'S.....

YOU'RE A RED BLOOD CELL (ERYTHROCYTE)! YOU'RE ALWAYS ON THE GO, HELPING OTHERS AND MAKING SURE EVERYTHING STAYS BALANCED. LIKE A RED BLOOD CELL, YOU'RE A VITAL PART OF THE TEAM, KEEPING THINGS FLOWING!

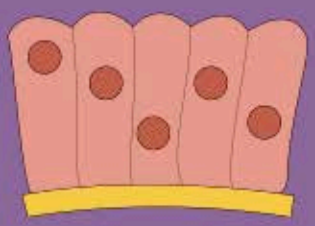


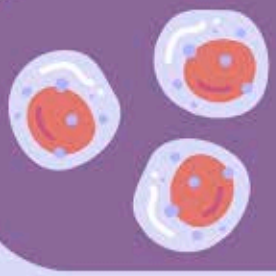
## IF YOU GOT MOSTLY D'S.....

YOU'RE A MACROPHAGE! YOU'RE A NATURAL DEFENDER, ALWAYS READY TO TACKLE CHALLENGES AND PROTECT THOSE AROUND YOU. LIKE A MACROPHAGE, YOU CLEAN UP THE MESS AND KEEP THINGS SAFE.

## IF YOU GOT MOSTLY E'S.....

YOU'RE AN EPITHELIAL (SKIN) CELL! YOU'RE THE ORGANIZED, PRACTICAL TYPE WHO KEEPS THINGS IN ORDER. LIKE AN EPITHELIAL CELL, YOU CREATE BOUNDARIES THAT HOLD EVERYTHING TOGETHER.



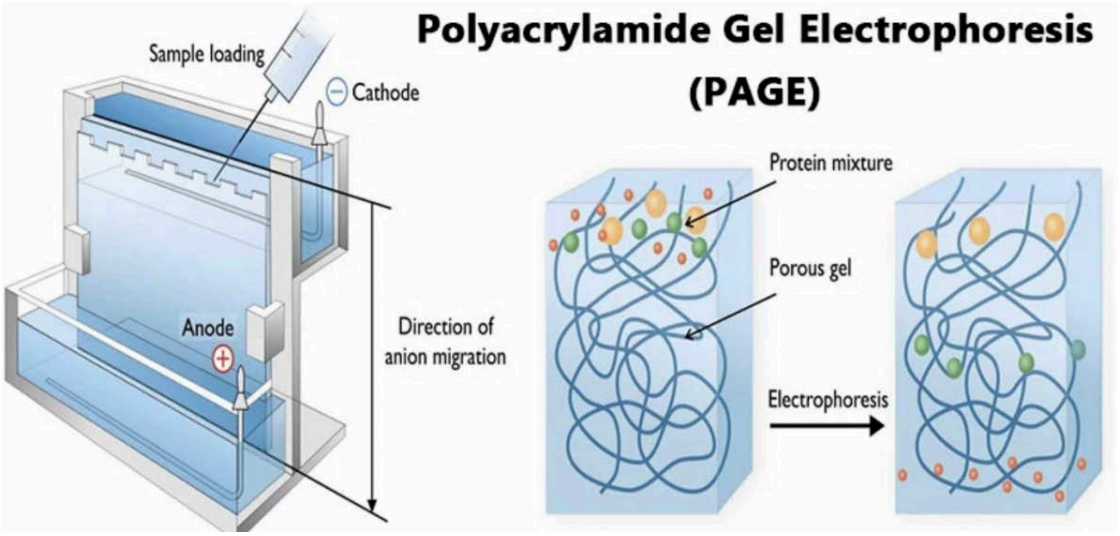


## IF YOU GOT MOSTLY F'S.....

YOU'RE A STEM CELL! YOU'RE ADAPTABLE AND VERSATILE, ABLE TO TAKE ON WHATEVER ROLE IS NEEDED. JUST LIKE A STEM CELL, YOU'RE THE ULTIMATE JACK-OF-ALL-TRADES!.

# EXPLORING POLYACRYLAMIDE GEL ELECTROPHORESIS

**ESTHER CHANG**
School of Molecular and Cellular Biology, University of Illinois Urbana-Champaign




Electrophoresis is a well-known technique among students in the life sciences. Most students learn how to run an agarose gel (a jelly-like gel infused with buffers), which allows the researcher to load DNA samples and observe their migration in hands-on lab experiments. However, there is another member of the gel electrophoresis family that may not be as familiar: polyacrylamide gel. Polyacrylamide gel electrophoresis is an essential tool in studying various biomolecules, including DNA, RNA, and proteins, supporting research fields such as biochemistry, biotechnology, and forensic chemistry. Unlike agarose gel, which can separate a broad range of nucleotide sizes and is relatively simple to set up, polyacrylamide gel is used for high-resolution separation of proteins and sometimes DNA or RNA, typically ranging from just a few base pairs to a few hundred. The methods and procedures for running this type of gel are different from the agarose gel, but they are incredibly useful!

**MATERIALS NEEDED**

**1. Electrophoresis chamber →** different one than agarose; this one is much larger

**2. Two glass plates →** required to form the gel mold

**3. 4 clips →** used to secure the plates together and prevent leakage when loading the buffer

**4. Urea →** included in denaturing gels

**5. Buffer →** maintains proper conditions for electrophoresis

**6. Power source →** supplies voltage for sample migration

**7. Micropipette →** for precise sample loading

**PROCEDURE**

**1. Prepare the gel solution**

a. Weigh out the required amount of urea (depending on the percentage of gel) and dissolve the solution at room temperature.

**2. Pour the gel**

a. Adjust the gel percentage based on the size of the fragments that are being analyzed.

**3. Set up the gel**

a. Carefully pour the liquid gel solution between the plates.
b. Avoid bubbles, which can affect sample migration.

**4. Allow the gel to polymerize**

a. Let it sit at room temperature for 45 minutes to 1 hour until it is fully polymerized and has a jelly-like structure.

**5. Prepare the gel lanes**

a. Gently remove the lane combs and rinse the wells carefully.
b. Skipping this step might lead to distorted results.

**6. Set up the gel for electrophoresis**

a. Secure the gel in the electrophoresis chamber and let it pre-run for about 30 minutes.

**7. Load the samples**

a. Always turn off the power source before loading the samples; otherwise, the samples will migrate prematurely.

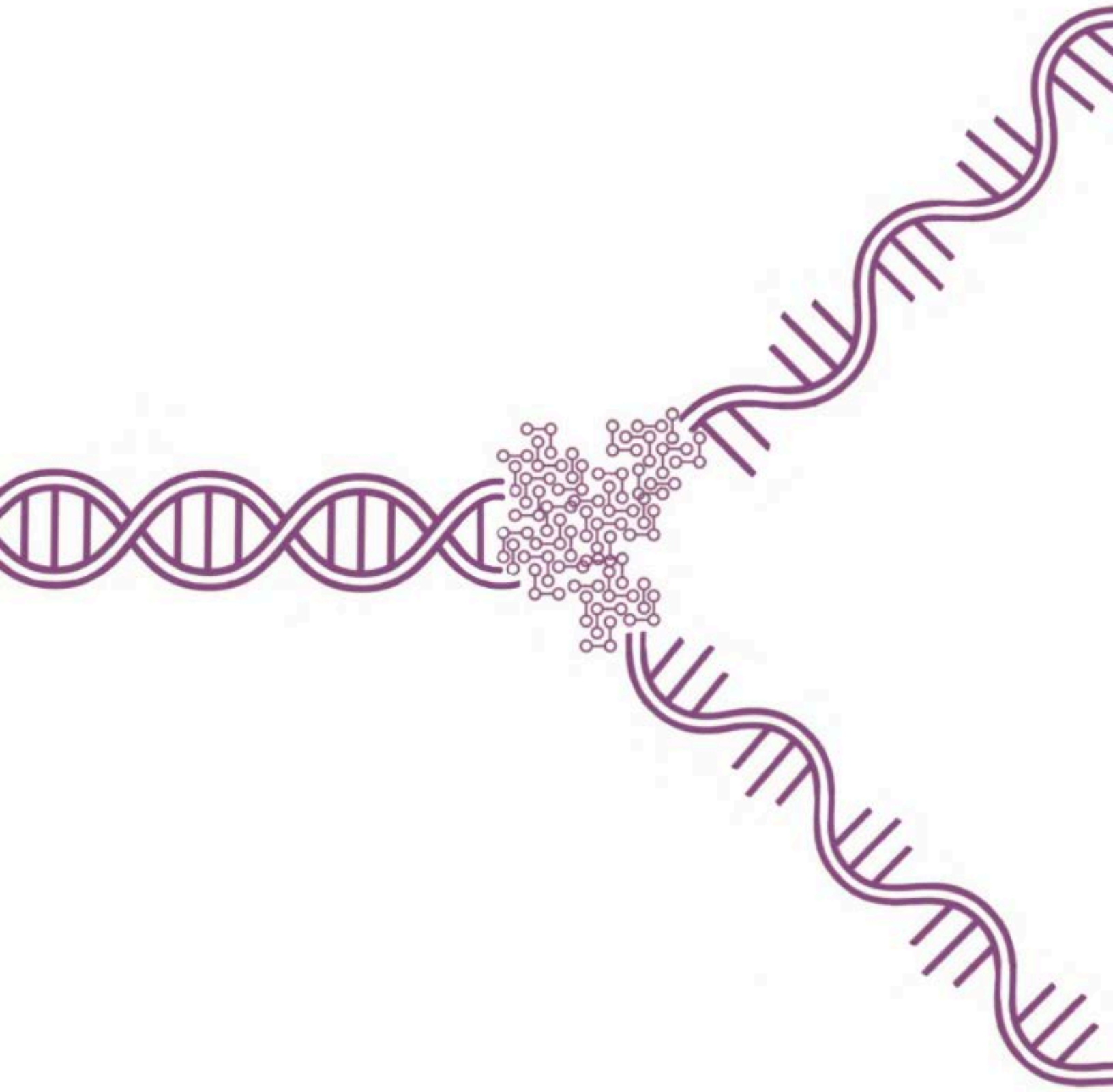
**8. Run the gel and observe migration**

a. The run time depends on the gel percentage.
b. A lower percentage requires a shorter amount of time, typically ranging between 1.5 to 2 hours.

Experiencing polyacrylamide gel electrophoresis for the first time can be truly fascinating. The technique requires practice and precision, but the beauty of gel migration and the information it provides are truly remarkable.

*Source/Image credits: Polyacrylamide gel: Overview & applications. Excedr. (n.d.).*

# THE TEAM



**CASEY MESKOVICH**
**President**

Casey is majoring in chemical and biomolecular engineering, and is on the biomolecular engineering track. She founded DHD as a way to share her passion for science. Outside of DHD, Casey works in the Sirk Lab, conducting research on the gut microbiome. After graduation she hopes to attend medical school.

**CAROLINA ORSO TONDO**
**Vice President**

Carolina is a junior majoring in molecular and cellular biology. She is currently working at Nanjappa Lab exploring fungal infections in immunocompromised subjects and hopes to continue conducting research after graduation. She also enjoys crocheting and traveling.

**MAYA KAFALI**
**Design Team Lead**

As a molecular and cellular biology major on the pre-medical track, Maya has always had a passion dedicated to the concepts of human and cellular functions. For this reason, she has dedicated her studies towards learning the hows and whys in these every-growing concepts, which brought her to Double Helix Digest. Combined with her additional passion in graphic design, she has found a community that pleasantly focuses on all things science, which eventually led her to the design team lead role. In this position, Maya has been able to combine the arts and sciences into leading a group of people with the same passions and talents. She hopes to carry these passions with me past college and to continue these efforts in future publications for DHD.

**ANDREW HAMILTON**
**Editor-In-Chief & General Editor**

Andrew is a junior with a major in neuroscience and minors in Spanish and chemistry. One thing he enjoys about editing is that he gets to read so many interesting articles about science-related discoveries every day! Outside of the club, he pursues research regarding optimization with on-tissue chemical derivatization.

**ALEXANDER WRIGHT**
**Treasurer**

Alex is majoring in molecular and cellular biology and has a minor in chemistry. He works as an EMT on campus and is on the pre med track with hopes of going to medical school after graduation. He also is on the executive board for two other RSOs and enjoys being a part of those groups. Outside of class he enjoys movies and snowboarding.

**BELEN RUBIO**
**Event Coordinator**

Belen is a sophomore majoring in integrative biology with a minor in chemistry. She joined DHD to explore a wider range of research fields and to refine her skills in scientific writing. Beyond her involvement in DHD, Belen is an active member of Beta Psi Omega where she serves on different committees and mentors incoming new members. In the future, Belen hopes to become a physician assistant and remain engaged with emerging research throughout her career.

**SOFIA ROSSETTI**
**Membership Director & Writer**

Sofia is a senior double-majoring in molecular and cellular biology and psychology. She is a research assistant in the SCOPE Neuroscience lab investigating both the cognitive and emotional aspects of the brain. She is a pre-med and she will be going to Italy to start her medical journey. In her free time she loves playing tennis, reading and traveling.

**MEGAN O'SULLIVAN**
**Assistant Editor-In-Chief & General Editor**

Megan just finished her sophomore year majoring in the integrative biology honors program and minoring in both Chemistry and Statistics. She chose to work as an assistant editor-in-chief because of her extensive background in writing, and her favorite part of the position is having a hand in shaping so many interesting articles to help each author's voice come through their work. As an editor, she strives to bring clarity to articles to help make science more accessible to the public, increasing both interest and understanding in the processes that can shape our everyday lives. Outside of the DHD, Megan also is interested in exploring the connections between ecology and environmental health.

**ANANYA SAMPATHKUMAR**
**Assistant Editor-In-Chief & General Editor**

Ananya is a sophomore, majoring in neuroscience with minors in chemistry and public health. With a background in writing and a passion for science, Ananya enjoys editing and serving as Assistant Editor-in-Chief, where she explores diverse topics and helps communicate complex ideas clearly and concisely. Outside of Double Helix Digest, Ananya is a writer for Brain Matters, a member of Star Course, a volunteer at Carle Hospital, and works at the Office of Undergraduate Admissions as a tour guide. In her free time, Ananya likes to read books, make jewelry, watch movies, and hang out with her friends.

**SHARON IGNATIUS NEWTON**
**Assistant Editor-In-Chief & General Editor**

Sharon is a junior studying bioengineering with a minor in computer science. She joined Double Helix Digest as both an editor and an assistant editor-in-chief to strengthen her scientific communication skills, gain deeper experience in the editing process, and help writers translate their research into clear, engaging writing. Sharon particularly enjoys providing one-on-one feedback to refine technical writing and make complex topics accessible. Outside of DHD, she conducts research in Dr. Sirk's lab and works as a lab assistant for a Bioengineering course. After graduation, she hopes to pursue a PhD in bioengineering and become a research professor.

**KAMARI HOWARD**
**General Editor**

Kamari is a senior graduating with a degree in integrative biology. In the future, she plans on becoming a wildlife conservationist and doing research to help endangered species. She decided to join DHD to be a part of the scientific community on campus and loved being able to read/review article topics students were interested in!

**RIYA SIVARAMAN**
**General Editor**

Riya is an incoming sophomore at the University of Illinois at Urbana-Champaign majoring in molecular and cellular biology with a minor in public health. She joined DHD to explore flexible research and gain experience in a field she's passionate about. As a pre-med student, Riya is interested in the origins of disease, cancer, and biopsychology. In addition to editing with DHD, she serves on the board of the American Medical Student Association on campus. She hopes to pursue a career in medicine in the future.

**BRISA SUN**
**General Editor**

Brisa is a sophomore at the University of Illinois majoring in biochemistry. She is currently involved in Dr. Shapiro's lab, focusing on the development of small molecule cancer therapeutics.As an editor, Brisa enjoys exploring a wide range of student work and collaborating with writers to strengthen their research and refine their ideas.

**FRAN AUSTRIACO**
**General Editor**

Fran is an editor for Double Helix Digest. They are a rising senior majoring in crop sciences, with a concentration in agroecology. Their research interest is in soil sciences and herbaria. Outside of academics, they enjoy drawing, designing, and cooking!

**KASANDRA MEDRANO**
**General Editor**

Kasandra is a sophomore studying molecular and cellular biology. She is currently on the pre-med track in hopes of specializing in surgery and possibly working with children! When she's not catching up on lecture videos, you can find her painting with watercolors or rewatching Gilmore Girls for the millionth time.

**POORVA KASTURE**
**Writer**

Poorva is a sophomore majoring in molecular and cellular biology. Poorva became involved with Double Helix Digest to learn more about the breadth of the field of biology and how different aspects can intertwine to come up with cohesive solutions, being especially interested in multidisciplinary research. In addition to writing for DHD, she is also involved in Beta Psi Omega, a professional fraternity for the biological sciences, and hopes to pursue a career in the medical field.

**KYRA MILLER**
**Writer**

Kyra is a freshman and from Glen Carbon, Illinois (near St. Louis, Missouri). She is studying integrative biology honors with minors in public health and chemistry in the hopes of attending medical school, a genetic counseling graduate program, or working in public health policy. Outside of academics, Kyra loves to read, listen to music, spend time in nature, bake, and go on late night sweet treat runs.

**IPSITA MANDAL**
**Writer**

Ipsita is a freshman majoring in molecular and cellular biology. She became involved with Double Helix Digest to have a space to explore her dual interest in psychology and genetics and also discover other potential interests. Outside of Double Helix Digest, Ipsita is also involved in research for Dr. Grosman's lab and volunteering as part of MEDLIFE.

**DHRUV TOMAR**
**Writer**

Dhruv is a sophomore majoring in integrative biology and computer science. He is interested in the applications of computational tools to shed new light in the field of biology, and plans to further pursue this interest in graduate school. Dhruv is currently working on research in the Van Doren Lab of Migration Biology and serves as the treasurer of the Illini Wildlife and Conservation Club. He became involved with the Double Helix Digest to help raise awareness of issues that threaten global bird populations.

**CLAIRE SKOWRON**
**Writer**

Claire is a sophomore at the University of Illinois studying chemistry and statistics. Some of her academic interests include health equity concerns, toxicology and time series analysis. She is involved in research labs across campus, all of which focus on different areas of public health such as disability accessibility, life history epidemiology and chronic pain management. On campus, she is also involved in MEDLIFE UIUC as the Director of Education and TEDxUIUC as the Director of Logistics. Outside of school, Claire enjoys playing viola, spending time outside and watching reality TV.

**ROBERT KEMP**
**Writer**

Robert is sophomore at the University of Illinois majoring in biochemistry. Robert joined Double Helix Digest because he is interested in learning about the biochemical mechanisms underlying human disease, pharmacology, and endocrinology. On campus, Robert studies enzymes in the biosynthetic pathway for a common chemotherapy agent in the Schuler lab. He is also the president of a social fraternity.

**ESTHER CHANG**
**Writer**

Esther is a sophomore majoring in biochemistry. She is interested in being a part of DHD because she wants to learn more about science and introduce more biology and chemistry-related content to the general public. She is also involved in research in Professor Silverman's lab. In the future, Esther hopes to pursue a PhD in chemistry.

**MANASWINI MANNAM**
**Writer**

Manaswini is a sophomore at the University of Illinois majoring in molecular and cellular biology. Manaswini joined Double Helix Digest to combine her passion for science and writing. Outside of being a writer for DHD, Manaswini is involved in nutrition research. In the future, Manaswini hopes to pursue a career in healthcare.

**ANDY GRANADOS**
**Writer**

Andy is a freshman bioengineering major at UIUC and a member of the Double Helix Digest club. Andy loves writing informatics pieces that bridge science and storytelling. On campus, he is immersed in soft-robotics research, where he helps build compliant actuator prototypes for K–12 education. This summer, Andy is assisting on a pressure-ulcer-relief wheelchair project, integrating sensor feedback and adaptive soft-actuator designs to improve user comfort. Down the road, Andy plans to pursue graduate studies in biomedical engineering and channel his skills into developing next-generation cancer-therapy devices.

**BOLADE ABIOLA-FAGBA**
**Writer**
Bolade is a freshman at the University of Illinois at Urbana Champaign under the Division of General Studies. In the future, she hopes to become a medical scientist. In her free time, Bolade enjoys reading and sleeping.

**AKSHATA GAJULA**
**Design Team**
Akshata is a sophomore at the University of Illinois, majoring in biochemistry. Akshata became involved in Double Helix Digest to learn more about medicine and contribute in a creative fashion. In addition to designing for DHD, Akshata is involved in various RSOs like Positive Pre-Meds and Global Medical Brigades. In the future Akshata hopes to go to medical school and become a doctor.

**NALI PATEL**
**Design Team**
Nali has just finished her freshman year as a molecular and cellular biology major at the University of Illinois at Urbana-Champaign.

**AND AN ADDITIONAL THANK YOU TO: Yunji Wan (Writer) Andy Ma (Writer) Lola Bahrs (Editor) Alison Ho (Editor) Layla Zarei (Editor) Micah Wang (Secretary)**

## GETTING INVOLVED

DHD IS A REGISTERED STUDENT ORGANIZATION WITH THE UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN, FOUNDED WITH THE INTENTION OF GUIDING UNDERGRADUATE STUDENTS THROUGH THE SCIENTIFIC WRITING PROCESS. FOR INQUIRIES ABOUT GETTING INVOLVED WITH DHD, PLEASE EMAIL @UIUCDHD@GMAIL.COM.