

Glowing Hope: How Bioluminescent Animals Are Illuminating The Fight Against Neurodegenerative Diseases

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ABSTRACT: BLI or bioluminescence imaging assists researchers in observing molecular and cellular mechanisms of living organisms as it allows visualization in real time and is non-invasive. This study focuses on using “glowing” mice and zebrafish which have been genetically modified to express luciferase or fluorescent proteins for monitoring neuroinflammation, protein aggregation, and neuronal activity. Antares-NanoLuc with cephalofurimazine is an example of advanced BLI that provides brain-penetrant, high-intensity signals which enable continuous monitoring of how a disease progresses over time and response to treatment. Furthermore, Alzheimer’s, Parkinson’s, Huntington’s diseases, and ALS have served as models for understanding the early pathological changes that occur, integrating drug-response studies, and advancing transnational research. In this paper, we discuss everything concerning animal testing and genetic modification from ethics and technicality to prospects such as CRISPR tagging, AI imaging. Bioluminescent modeling as a whole provides new opportunities to study neurodegeneration as new understanding of mechanisms through dynamic observation sensitivity is made possible alongside devising new therapeutic approaches.

KEYWORDS: Bioluminescent Animals, Alzheimer's Diseases, Zebra Fish

INTRODUCTION

Biological processes in living animals can be seen with remarkable sensitivity and very little background noise using bioluminescence imaging (BLI), which uses the natural light-emitting reaction between luciferase enzymes and small-molecule luciferins. Modern BLI, which has its origins in fireflies, marines, and

bacteria, has been refined for use in neurological applications by creating designer luciferase–luciferin couples that are more vivid, red shifted, and able to pass through the blood–brain barrier. It is now possible to track molecular and cellular events, such as neuro inflammation, protein aggregation, and neuronal activity, in live rodents with high resolution and

longitudinality thanks to the recent introduction of substrates like cephalofurimazine, which, when combined with Antares or NanoLuc, produce brain signals that are more than 20× stronger than before invasive imaging, which makes BLI a crucial tool for looking into the course of neurodegenerative diseases and assessing treatment plans in vivo (1-4).

Bioluminescence is not just a phenomenon; it's a reality. Deep diving into the ocean where aquatic life emits ethereal glows imagine that same light now directing researchers to examine the living brain. In live animal models of Alzheimer's, Parkinson's, ALS, and other diseases, researchers can non-invasively monitor neuroinflammation, protein aggregation, and neural activity using bioluminescence imaging (BLI), which is made possible by enzymes known as luciferases and their illuminating molecules, known as luciferins. The discovery of cephalofurimazine (CFz) in combination with Antares-NanoLuc luciferase, which penetrates the blood–brain barrier and generates signals more than 20 × brighter than conventional systems, marked a significant advancement and allowed for real-time, video-rate imaging of brain activity in freely moving mice. The possibilities are endless: in injury and illness models, bioluminescent

optogenetics, which combines luciferase with light-sensitive proteins like luminopsins, is facilitating internal neuronal activation to encourage axon regeneration . It is a ground-breaking instrument that is shedding light on how we understand and treat neurodegenerative illnesses, thus this live light is not fiction.

Researchers have created genetically modified "glowing" animal models, usually mice, that express luciferase under disease-relevant promoters. This enables non-invasive, real-time monitoring of neurodegenerative illnesses such as Huntington's, Parkinson's, Alzheimer's, and ALS. Bigenic mice that are designed to manufacture human mutant APP and express luciferase driven by the GFAP promoter, for instance, show age-dependent increases in bioluminescent signal in Alzheimer's research. These increases closely correspond with the formation of β amyloid plaque. These signals allow for quantitative, long-term tracking of the course of the disease and the effectiveness of treatment in the same animal. They can be detected as early as 7 months in CRND8 crosses and 14 months in APP23 crosses. Researchers can also use tauopathy models, like the P301S GFAP-luciferase mice, to see tau aggregation and astrogliosis by sequential imaging. These models offer real-time evaluation of treatments like

EPO-derived peptides and prediction indicators of neurological impairment . Huntington's and Parkinson's disease models are also often employed because they provide platforms for investigating molecular causes and testing treatments. These models show hallmark protein aggregation and neuronal death in mice that express enlarged huntingtin or mutant α synuclein. Meanwhile, human motor neuron degradation is mirrored in ALS mice that overexpress mutant SOD1, allowing for visual tracking of the onset and progression of the disease . By facilitating longitudinal, minimally intrusive experiments within living organisms, these cutting-edge bioluminescent animal systems collectively revolutionize our capacity to uncover hidden disease processes, speed up medication discovery, and improve our comprehension of neurodegeneration (5-8).

Alzheimer's, Parkinson's, ALS, and Huntington's diseases together impact hundreds of millions of people worldwide; dementia alone affects 55 million people today and is expected to reach 150 million by 2050 . Neurodegenerative diseases represent a rapidly growing global health crisis. In 2021, neurological illnesses—such as strokes, dementia, and Parkinson's disease—accounted for 443 million disability-adjusted life years (DALYs) and

11.1 million deaths, or nearly 43% of the world's population . These disorders are now the main cause of illness and disability. Over 10 million individuals currently suffer from Parkinson's disease, and estimates indicate that number will rise to over 12 million by 2040. The disease's prevalence alone has increased by more than 280% since 1990. Because of these concerning trends—which are brought on by aging populations, shifting lifestyles, and health disparities—new research methodologies that can track disease processes, test treatments, and speed up discoveries are needed. Non-invasive, longitudinal, real-time insights into mechanisms such as protein aggregation, neuro inflammation, and neuronal dysfunction are precisely what genetically modified, bioluminescent animal models offer. These innovative instruments are essential for preventing these illnesses from worsening their economic and social costs.

THE SCIENCE OF GLOWING ANIMALS

In the amazing natural phenomenon known as bioluminescence, organisms produce visible light by means of a chemical interaction. The molecules known as luciferin are at the core of this "cold light" process; when oxidized by the enzyme luciferase in the presence of oxygen, they

produce a softly glowing light without producing heat. Around 75–90% of deep-sea organisms, such as fish, jellyfish, squid, and plankton, generate light, frequently in a blue-green color that moves through water efficiently. This adaptation is particularly common in the ocean .

On land, creatures like glowworms, fireflies, and bioluminescent mushrooms illuminate the night sky; these organisms typically use warmer hues, like yellow or green, to blend in with their terrestrial surroundings. Bioluminescence has a long and varied evolutionary history. It has developed on its own more than 40 times, and the earliest evidence of it was discovered in soft corals some 540 million years ago. This suggests that light signaling is one of the earliest survival mechanisms on Earth (9-12).

In addition to being aesthetically pleasing, bioluminescence has important ecological roles. Deep-sea animals use it to blend in with their surroundings, attract prey, or fend off predators. Accordingly, fungi light to attract insects that spread their spores, whereas fireflies flash to attract mates. The luciferase enzymes are now widely employed in biotechnological and medical research to measure gene expression, analyze cell behavior, and even construct living biosensors. It is remarkable that this natural light has also spurred human

invention. To put it succinctly, bioluminescence is a marvel of the inventiveness of nature, turning basic chemistry into amazing light displays that reveal the hidden facets of life and spark new scientific research.

Although bioluminescence and biofluorescence both produce enthralling glows in the natural world, their underlying processes are very different and differences are tabulated in Table 1. An enzyme called luciferase oxidizes a substrate called luciferin, frequently with oxygen, releasing energy in the form of visible light. This process is how living things make bioluminescence, a self-generated light. Without the need for outside lighting, organisms such as fireflies, anglerfish, illuminating mushrooms, and bioluminescent bacteria employ this "cold light" for defense, communication, or luring prey. Contrarily, biofluorescence happens when an organism absorbs light, typically blue or ultraviolet, and instantly re-emits it at a longer wavelength, only glowing when exposed to external light. Only photon absorption and emission are involved; no chemical reactions take place. Certain amphibians, birds, corals, scorpions, and even certain mammals exhibit this phenomenon.

While bioluminescence is a chemical process that produces light without the aid

of ambient light, bio fluorescence is a physics-based glow that requires external

light to function and ends as soon as the illumination is turned off.

Table 1. Difference between Bioluminescence & BioFluorescence

Feature	Bioluminescence	Bio fluorescence
Light source	Internal chemical reaction	External light source (e.g. UV/blue)
Heat produced	Virtually none ("cold light")	None (reemission of absorbed photons)
Dependence	No external light required	Must be illuminated externally
Examples	Fireflies, anglerfish, glow worms	Scorpions, corals, some amphibians

A biological process known as bioluminescence occurs when living things produce their own light via an internal chemical reaction. Usually, an enzyme called luciferase oxidizes a substrate called luciferin, frequently with the help of oxygen and cofactors like ATP. The result is an excited molecule, such as oxyluciferin, which produces light as it decays back to its ground state. Because of its tremendous efficiency and nearly zero heat production, this reaction is frequently referred to as "cold light." From terrestrial species like fireflies and glowworms to marine animals like bacteria, jellyfish, and deep-sea fish, bioluminescence is found in a wide variety of life forms. Prey attraction, defense, mating signals, and camouflage through counter illumination are just a few of its many biological functions. In situations where cell density is high, several

bacteria even use quorum sensing to control how much light they produce.

The physical process of bio fluorescence, on the other hand, occurs when organisms absorb light from the environment, usually blue or ultraviolet wavelengths, and then instantly reemit it at a longer wavelength, giving the appearance of a glow in hues like orange, red, or green. When the excitation light is turned off, this phenomenon stops and doesn't require any internal chemical reactions. The effect aids animals with tasks like UV protection, communication, and camouflage. The chemicals that cause this are fluorescent proteins or other biomolecules (e.g., GFP in jellyfish). Numerous taxa, including corals, jellyfish, fish, sharks, certain amphibians, birds, and even mammals like platypus and flying squirrels, have been shown to exhibit bio

fluorescence, however its ecological functions are still being studied (13-15).

GLOWING ANIMALS AID RESEARCH INTO NEURODEGENERATIVE DISEASES

Because of their genetic tractability, transparency, quick life cycles, and biological relevance to humans, a number of important model organisms are frequently utilized in bioluminescence and fluorescence research. For mammalian research, mice (*Mus musculus*) are the gold standard. Researchers can see gene expression and disease progression in vivo, for example in cancer and developmental studies, by using genetically modified mice that express luciferase or fluorescent proteins like GFP, YFP, or mCherry. Due to their transparent embryos, analogous orthologs for about 70–85% of human disease genes, and ease of genetic manipulation, zebrafish (*Danio rerio*) are particularly common in fluorescence imaging and bioluminescence assays to track cellular activity, organ development, and xenografted tumors in real time. Around 75% of human disease genes have functional counterparts in flies, which makes them perfect for fluorescent tagging and luciferase reporters in quick, high-throughput investigations of gene function and signaling pathways. This makes the

fruit fly (*Drosophila melanogaster*) one of the most useful invertebrate models for genetic and developmental research. Similar to this, the nematode worm (*Caenorhabditis elegans*) offers an optically transparent system throughout its life cycle, making it possible to track neural or developmental processes in vivo and image GFP fluorescence in single cells in real time. More than 60% of the genes linked to human diseases are orthologs in the worm. Zebrafish combine vertebrate complexity with live imaging feasibility, fruit flies and worms allow for quick, large-scale genetic and fluorescent screening, and mice provide physiological relevance in mammals. These model organisms make up a complementary research toolkit that has been used in bioluminescent or biofluorescent studies to shed light on cellular activity, developmental pathways, and disease mechanisms.

In neurodegenerative research, bioluminescent "glowing animal" models are essential because they allow for the non-invasive, real-time viewing of disease-related molecular and cellular processes in living things. Bigenic animal models of Alzheimer's disease, such as Tg(CRND8 luc) and Tg(APP23 luc), for instance, express luciferase in response to the GFAP promoter, an astrocyte marker. These animals accurately mimic the evolution of

pathology by emitting light in response to luciferin injections, which correlate to astrocyte activation and the production of amyloid- β plaque. Signals in the brain rise at 7 and 14 months of age, respectively. By using this method, scientists can monitor the progression of a disease over time in individual animals, providing a quick and quantifiable substitute for behavioral testing or post-mortem examination

Likewise, in models of Parkinson's disease, mice that have been genetically modified to express human α -synuclein in neurons in conjunction with astrocyte-driven luciferase allow for non-invasive assessment of neuroinflammation, identifying astrogliosis before motor symptoms and removing the requirement for invasive histopathology. Additionally, tauopathy models, like P301S mice crossed with GFAP-luc reporters, have benefited from bioluminescence imaging, which measures reactive astrocytes over time and evaluates the effectiveness of treatments like EPO-derived peptides. Signal trends in bioluminescence imaging are in line with gliosis and tau pathology.

One recent development is the application of sophisticated luciferase substrates, such as AkaLumine, in Alzheimer's disease models. This technique, when paired with near-infrared luciferases, may directly bind

and report amyloid- β aggregation, providing increased sensitivity and a good association ($R^2 = 0.81$) with plaque load and in vivo therapy response. In neurodegenerative research, bioluminescence imaging is a transformative tool for understanding tauopathies, Alzheimer's, Parkinson's, and related disorders because it provides high sensitivity, quantitative tracking, and the ability to track disease trajectories and treatment effects in the same living subjects.

Our understanding of neurodegenerative disorders has greatly advanced as a result of researchers' development of potent imaging tools that enable real-time observation of protein aggregates within living creatures, such as tau tangles and amyloid- β plaques. Luminescent conjugated oligothiophenes (LCOs) are one such technique. These molecules easily penetrate the blood-brain barrier and attach to aggregated proteins, illuminating them for multiphoton microscopy longitudinal fluorescent imaging. As an example, the LCO variation HS 84 has made it possible to observe the creation of tau tangles in real time, while HS 169 has been used to visualize amyloid β in both red and green spectral modes, which has allowed researchers to trace the evolution of the disease over time from a single injection. In addition, the use of high-

end fluorescence microscopy to record the dynamics of amyloid-beta oligomerization and fibrillization in real time at single-molecule resolution has been made feasible using quantum dot-labeled A β peptides (QDA β). These investigations allowed for the imaging of microglial uptake of amyloid species and observed aggregation dynamics, including lag period, growth phase, and saturation, in living cells. Crucially, in Alzheimer's research, bioluminescence imaging (BLI) has become a dependable quantitative, non-invasive technique. Using AkaLumine, a near-infrared luciferase substrate that selectively binds to amyloid aggregates, is a recent breakthrough. AkaLumine is buried by amyloid plaques early on and released later in transgenic mice expressing AkaLuciferase. This results in real-time light signals that closely correlate ($R^2 \approx 0.81$) with actual A β burden and therapy outcomes. Simultaneously, previous models that employed GFAP-driven luciferase under astrocyte activation reliably reflected A β deposition patterns, achieving stage-specific BLI signal increases at around 7 months in Tg(CRND8 luc) and 14 months in Tg(APP23 luc) mice.

When combined, these sophisticated luminescent and fluorescent probes offer researchers a sensitive, dynamic, and non-invasive window into the temporal and

spatial evolution of pathological protein aggregates. This allows for quantitative evaluation of aggregation kinetics, single-subject longitudinal studies, and real-time monitoring of the effectiveness of treatments in models of neurodegenerative diseases.

Researchers can now observe the non-invasive, dynamic, and quantifiable degradation of brain cells over time using bioluminescent imaging techniques, which are essential for monitoring the course of neurodegenerative diseases and the emergence of symptoms in living animal models. According to BioMed Central, bioluminescence reporters linked to the GFAP gene show astrocyte activation in the brain and spinal cord as early as 3 days post-induction in mouse models of multiple sclerosis, including experimental autoimmune encephalomyelitis (EAE). This is well before any motor symptoms start to show up around 10 days later, and bioluminescence levels by day 14 strongly hint at clinical severity. Likewise, in Alzheimer's models, bigenic animals that express luciferase under GFAP exhibit a distinct age-dependent rise in signal intensity: astrocyte activation begins at approximately 7 months for Tg(CRND8) mice and 14 months for Tg(APP23) mice, closely resembling the buildup of amyloid β plaque. This makes it possible to track a

disease over time in the same people without invasive treatments.

In addition to monitoring glial activation, scientists have employed Dcx-Luc reporter mice to track neurogenesis and blood–brain barrier integrity as the disease progresses, such as following Parkinson's-related injury. These mice have shown images of BBB breakdown and increased bioluminescence in the hippocampus. As reactive gliosis has been quantified over time in tauopathy models like as P301S mice, transgenic GFAP-luc constructions have made it possible to assess new treatments such EPO-derived peptides. Non-invasive, measurable, and longitudinal, these methods have several distinct advantages. They enable imaging of brain pathology, including inflammation, neuron loss, demyelination, and protein aggregation, before overt symptoms appear. By mapping the entire illness chronology in individual animals, this timely information helps researchers better understand neurodegeneration and explore early-stage therapies.

In neurodegenerative research, bioluminescent and fluorescent markers have transformed drug efficacy testing by enabling researchers to watch the neural effects of potential treatments in real time in living animals. For example, human

glioma cells that have been engineered to express firefly luciferase (e.g., U87MG-luc) are implanted intracranially in glioblastoma models. Serial BLI is a non-invasive method of tracking tumor burden and response to anti-angiogenic agents, and it has a strong correlation with MRI-based tumor volume and treatment effect. The evaluation of therapeutics is also aided by transgenic neurodegeneration models that express reporters like GFAP luc. For example, in P301S tauopathy mice, substances like EPO-derived peptides (like JM4) decrease gliosis-driven bioluminescence, enabling researchers to track neuroinflammatory reactions to treatment over time (16-19).

Neuronal damage dynamics are revealed by bioluminescent Smad luc reporters in neurotoxicity and injury models. Following kainic acid-induced excitotoxicity, BLI rises within 12 hours, and the administration of neuroprotective medications such as MK 801 dramatically reduces this signal, indicating the in vivo efficacy of the agent. Similarly, MITO-Luc mice demonstrate hyperbilirubinemia-induced neurotoxicity and the reduction of BLI signals by treatments such as minocycline or bevacizumab, indicating the effect of treatment on cell proliferation.

Bioluminescence is also used to assess transplanted cell therapies: luciferase-tagged neural stem cells are injected into models of Parkinson's disease, and their survival is monitored over a period of days to weeks, offering a quantitative assessment of the treatment's viability and effect on motor function .

Glowing indicators are useful for a variety of drug testing applications, including the non-invasive measurement of disease progression, the assessment of neuroprotective or anti-inflammatory effects, the evaluation of cell transplant viability, and the long-term screening of gene/cell therapies in the same living patients. This results in a more effective, compassionate, and perceptive assessment of neurodegenerative treatments.

By using CRISPR/Cas9 gene editing, scientists have been able to precisely and in real time analyze neurodegenerative pathways under physiological regulation by directly inserting glowing tags, like as fluorescent proteins or luciferase, into endogenous disease-related genes. In human cell lines, tagging the SNCA gene (which codes for α -synuclein) with NanoLuc luciferase at its 3' end is a prominent example of Parkinson's disease research. In contrast to artificial, promoter-driven reporters, this CRISPR-engineered system efficiently tracks α -synuclein

transcription within its natural epigenetic context and reacts to medication treatments . Using CRISPR insertion to fuse a destabilized luciferase to endogenous α -synuclein, an enhanced high-throughput screening cell line was produced. This results in shorter-lived luminescent signals that are perfect for effective drug screening and efficiently detecting changes in protein levels during brief assays. Researchers can now visualize protein expression by fluorescence or luminescence at physiological levels thanks to CRISPR, which has made it possible to integrate dual reporters, such as GFP and HiBiT tags, under the control of endogenous regulatory elements in neuronal cells and iPSCs. This is perfect for studying the effects of gene dosage or protein aggregation in Parkinson's, Alzheimer's, and related disorders . In addition to providing a strong platform for drug discovery and mechanistic insights into neurodegeneration, these CRISPR + glowing tag strategies offer high sensitivity, real-time tracking, and quantitative assays of disease-driving proteins. This allows for dynamic observation of how genetic mutations, epigenetic modulation, or therapeutic interventions affect pathologic protein behavior (20-22).

REAL-WORLD APPLICATIONS AND BREAKTHROUGHS

Glowing animals, genetically engineered to produce fluorescent or bioluminescent proteins, have opened up exciting new possibilities in the battle against neurodegenerative disorders. These models literally light up what's going on in the brain, allowing scientists to see unseen processes like the accumulation of hazardous proteins or the gradual decay of neurons in real time. This exceptional visibility has aided scientists in determining how illnesses such as Alzheimer's and Parkinson's advance—and, more crucially, the way to sluggish or prevent them. Thanks to these luminous creatures, what was previously hidden deep within the brain is now guiding the creation of real-world therapies.

ZEBRA FISH MODELS GLOWING TO TRACE TAU PROTEINS IN DEMENTIA:

Expressing fluorescent proteins, genetically modified zebrafish have become a practical model for studying the pathophysiology of neurodegenerative diseases including tauopathy-related illnesses like Alzheimer's disease and frontotemporal dementia. In these models, tau proteins are tagged with fluorescent markers like green fluorescent protein (GFP), which enables researchers to see and trace tau's expression, misfolding, aggregation, and propagation in real time

within living organisms . Normally stabilizing neuronal microtubules, tau proteins turn dysfunctional in dementia and produce dangerous aggregates that impair neuronal communication and hasten cognitive deterioration. Because of their transparency, which permits noninvasive monitoring of tau dynamics under fluorescence microscopy , zebrafish embryos and larvae are appropriate for longitudinal studies of these processes. These fluorescent zebrafish models provide many advantages, including real-time monitoring of tau propagation between neurons, evaluation of neuronal damage and loss over time, high-throughput screening of drug candidates targeting tau aggregation or clearance, and visualization of early tau pathology and progression into neurodegeneration. Furthermore, zebrafish's genetic and neurochemical similarities to humans and their fast development and cost-effective make them a feasible alternative to mammalian models in early dementia research .

GLOWING MICE HELPING DEVELOP EARLY DETECTION TOOLS:

Genetically modified luminous mice that express fluorescent or bioluminescent markers have been shown to be effective models for the early detection of neurodegenerative illness with Alzheimer's and Parkinson's. These transgenic mice

give crucial information on the onset and progression of these illnesses by allowing researchers to observe minor molecular and cellular changes that occur before the expression of clinical symptoms. Researchers can perform noninvasive imaging to observe protein misfolding, aggregation, and distribution throughout the brain in real time by tagging pathological proteins such as amyloid beta (A β), tau, or α synuclein with fluorescent markers such as GFP, YFP, or RFP. These visual cues facilitate early detection of proteinopathy, synaptic dysfunction, blood-brain barrier breakdown, and neuroinflammation associated with oxidative stress, prior to neuronal death. These models are particularly useful for validating diagnostic biomarkers like PET ligands and developing cutting-edge diagnostic technologies such as biosensors and molecular imaging devices. They also enable continuous monitoring of disease advancement and evaluation of therapeutic responses in the early phases. The 5xFAD mouse model, which expresses human genes linked to familial Alzheimer's disease mutations, is a well-known example. When combined with fluorescent reporters, it enables researchers to utilize in vivo two-photon microscopy to monitor amyloid plaque formation and microglial activation. Similarly, bioluminescent imaging has been utilized to detect early gene

expression changes linked with neuronal stress in tauopathy and ALS models. In general, glowing mouse models are critical for connecting basic molecular research with clinical imaging, facilitating the development of noninvasive early detection techniques for human neurodegenerative disorders.

Genetically modified glowing animals, including mice and zebrafish engineered to express fluorescent or bioluminescent markers, have played a pivotal role in identifying and advancing drug candidates into clinical trials for neurodegenerative diseases. These models allow real-time visualization of key pathological events, such as amyloid-beta (A β) aggregation, tau misfolding, and neuroinflammation, facilitating the early-phase screening and validation of therapeutic compounds. For instance, transgenic fluorescent mouse models like 5xFAD and APP/PS1, which develop A β plaques, were used extensively to assess the efficacy of monoclonal antibodies including aducanumab, lecanemab, and donanemab. These antibodies demonstrated the ability to reduce A β burden in these models before progressing to human clinical trials. Similarly, fluorescent zebrafish models, due to their optical transparency and rapid development, have been employed in high-throughput drug screening to identify

compounds that target tau aggregation, oxidative stress, and neurotoxicity (23-25). Promising hits from zebrafish screens, including kinase inhibitors and autophagy enhancers, have advanced to preclinical development and are being considered for future clinical testing. These glowing animal models provide a crucial bridge between basic science and therapeutic innovation, helping to de-risk drug candidates before they enter costly and complex human trials.

ETHICAL AND TECHNICAL CONSIDERATIONS: ADDRESSING CONCERNS ABOUT GENETIC MODIFICATION AND ANIMAL TESTING

The use of genetically modified animals in scientific research brings with it a range of ethical and technical challenges. From an ethical standpoint, altering the genetics of sentient organisms for experimental objectives raises serious concerns about animal suffering and humane treatment. Modifications such as inducing fluorescent protein expression or creating disease-prone animal models can unintentionally cause health complications or distress. To address these concerns, researchers follow the 3Rs principle—Replacement, Reduction, and Refinement—which promotes using alternatives to animal

testing, minimizing the number of animals used, and improving experimental procedures to reduce harm. Additionally, the ethical debate extends to the use of genetically modified animals in commercial settings, such as glowing pet fish, which has sparked public concern over the commercialization of living beings. There is also anxiety about the ethical justification of animal use when non-animal methods—like simulations, organoids, or lab-grown tissues—are increasingly available and effective.

On the technical side, the process of genetic engineering is complex and requires precise techniques to ensure that foreign genes are inserted and expressed accurately without negatively affecting the animal's normal biological functions. While fluorescent and bioluminescent models are valuable for in vivo imaging, their signals may weaken over time or be affected by tissue depth, photobleaching, or physiological interference. Bioluminescent imaging systems often require chemical substrates, which add another layer of complexity to experimental setups. In addition, maintaining colonies of genetically modified animals requires specialized infrastructure, significant funding, and technical expertise. Environmental concerns are also prominent, particularly the possibility of modified animals

escaping into the wild, potentially disturbing ecosystems or enabling gene flow into natural populations. Because of these risks, regulatory compliance, ethical oversight, and strict biosafety protocols are essential to ensure that such research is conducted responsibly. Overall, ethical sensitivity and technical precision must go hand-in-hand to ensure the responsible use of genetic modification and animal testing in science.

THE IMPORTANCE OF ETHICAL OVERSIGHT AND MINIMIZING HARM IN ANIMAL RESEARCH:

Obtaining ethical approval is a fundamental step in any research involving animals, ensuring that the study is designed and conducted responsibly, with proper regard for animal welfare. Researchers are required to submit their protocols to ethical review bodies such as the Institutional Animal Ethics Committee (IAEC) or the Institutional Animal Care and Use Committee (IACUC), depending on the region. These committees evaluate whether animal use is scientifically justified, and whether measures are in place to reduce potential harm. The widely accepted 3Rs framework—Replacement, Reduction, and Refinement—serves as a foundation for ethical animal research. "Replacement" emphasizes using alternatives to animals

when feasible; "Reduction" involves limiting the number of animals used without compromising the quality of results; and "Refinement" focuses on modifying procedures to ease pain, distress, or suffering. Implementing these principles not only supports animal welfare but also enhances the quality and reliability of scientific findings, as stressed or unhealthy animals can skew results (26-28).

CHALLENGES OF USING ANIMAL MODELS IN HUMAN BIOMEDICAL RESEARCH:

Although animal models have played a crucial role in biomedical discoveries and therapeutic development, they possess inherent limitations when it comes to replicating human biology. Species like mice, rats, and zebrafish are frequently used due to their ease of genetic manipulation and short reproductive cycles, but they differ significantly from humans at genetic, physiological, and biochemical levels. These differences can affect how diseases manifest and how treatments are processed, often leading to inconsistencies between animal study results and human clinical outcomes. For instance, numerous drugs that appear promising in animal models ultimately fail in human trials because of variations in immune function, metabolism, or disease progression. In the case of

complex conditions such as Alzheimer's disease, animal models often replicate only isolated features of the illness, failing to capture the full range of human-specific symptoms and disease mechanisms. These limitations underscore the need to complement animal models with alternative platforms like human-derived organoids, lab-on-a-chip systems, and in silico simulations. Blending traditional animal research with human-based technologies may improve predictive accuracy and promote more ethical and effective biomedical research.

THE FUTURE OF GLOWING RESEARCH

The future of glowing research is being shaped by the integration of advanced technologies such as CRISPR gene editing, artificial intelligence (AI), and real-time imaging techniques. These innovations enable the development of transgenic animals that express fluorescent or bioluminescent markers in specific tissues or under certain conditions, allowing researchers to study dynamic biological processes non-invasively. Coupling glowing models with AI and high-resolution microscopy has enhanced data interpretation, disease modelling, and drug discovery. Additionally, the use of glowing proteins in organoids and organ-on-chip systems is helping reduce reliance on

whole-animal models while improving human-specific insights. However, ethical considerations, such as animal welfare and ecological safety, will continue to demand rigorous oversight and responsible innovation.

NEXT-GENERATION TOOLS: COMBINING FLUORESCENCE WITH AI, 3D BRAIN MAPPING, AND REAL-TIME IMAGING:

Emerging technologies are revolutionizing the use of fluorescent tools in biological research by integrating them with next-generation techniques such as artificial intelligence (AI), 3D brain mapping, and real-time in vivo imaging. Fluorescent proteins, long used to visualize molecular processes, are now being leveraged alongside machine learning algorithms to analyse vast imaging datasets more accurately and efficiently than ever before. AI-driven image recognition and segmentation enable automated tracking of cellular activity and disease progression, reducing human error and accelerating data interpretation. Meanwhile, 3D brain mapping technologies, such as light-sheet fluorescence microscopy and tissue clearing methods like CLARITY, allow researchers to visualize entire neural networks in unprecedented detail within intact brain tissues. These tools have been particularly transformative in neuroscience, where combining glowing markers with

spatial transcriptomics enables the creation of functional brain atlases in animal models. Real-time imaging advances further enable scientists to monitor live biological processes—such as synaptic transmission, cancer metastasis, or immune cell behaviour—over time with minimal invasiveness. The fusion of these technologies provides a multi-dimensional understanding of biology, from single cells to entire organ systems, and holds promise for improving disease modelling, diagnostics, and precision medicine.

Genetically engineered glowing animals have become valuable tools in improving early disease detection and the development of more precise treatments. These models, which express fluorescent or bioluminescent proteins, allow scientists to visualize biological processes that are otherwise invisible, enabling the monitoring of disease onset and progression in real time. For instance, fluorescent imaging has helped detect tumors at very early stages—often before physical symptoms appear—allowing researchers to test targeted therapies more effectively (29-31). In the field of neurodegenerative disease, transgenic zebrafish and mice engineered to express human proteins linked to disorders such as Alzheimer's have made it possible to observe the early accumulation of toxic proteins like tau and amyloid-beta. This has

opened up opportunities for early intervention and therapeutic development. Additionally, glowing models facilitate high-throughput screening of drug candidates by enabling rapid and non-invasive assessment of biological responses. Altogether, glowing research is helping to close the gap between laboratory discoveries and real-world clinical solutions (32-44).

CONCLUSION

The use of luminescent imaging combined with genetically modified animal models has transformed research on neurodegenerative diseases. These glowing animal models allow non-invasive and real-time tracking of important pathological processes like the activation of astrocytes, amyloid-beta deposition, tau protein aggregation, and breaches in the blood-brain barrier. These models are already helping to preclinically validate some promising therapies and are leading us towards precision medicine. Ethically, and from a translational perspective, there are limitations these models face which need addressing. With the continued development of imaging substrates, gene editing tools like CRISPR/Cas9, and genetic reporters, the translational impact, as well as the sensitivity and specificity, will continue to grow. As we develop more

advanced systems that integrate bioluminescence with real-time data, analytics, AI, and models that are more human-relevant, research stemming from glowing beings will undoubtedly illuminate the path to solving puzzles surrounding neuro degeneration and expedite crafting robust treatments.

REFERENCES

1. Townsend KM, Prescher JA. Recent advances in bioluminescent probes for neurobiology. *Neurophotonics*. 2024 Apr 1;11(2):024204-.
2. Su Y, Walker JR, Hall MP, Klein MA, Wu X, Encell LP, Casey KM, Liu LX, Hong G, Lin MZ, Kirkland TA. An optimized bioluminescent substrate for non-invasive imaging in the brain. *Nature chemical biology*. 2023 Jun;19(6):731-9.
3. English AW, Berglund K, Carrasco D, Goebel K, Gross RE, Isaacson R, Mistretta OC, Wynans C. Bioluminescent optogenetics: A novel experimental therapy to promote axon regeneration after peripheral nerve injury. *International journal of molecular sciences*. 2021 Jul 5;22(13):7217.
4. Watts JC, Giles K, Grillo SK, Lemus A, DeArmond SJ, Prusiner SB. Bioluminescence imaging of A β deposition in bigenic mouse models of Alzheimer's disease. *Proceedings of the National Academy of Sciences*. 2011 Feb 8;108(6):2528-33.
5. Dunn-Meynell AA, Dowling P, Marchese M, Rodriguez E, Blumberg B, Choi YB, Gaindh D, Lu W. In vivo bioluminescence imaging used to monitor disease activity and therapeutic response in a mouse model of tauopathy. *Frontiers in Aging Neuroscience*. 2019 Sep 12;11:252.
6. Lee MK, Borchelt DR, Wong PC, Sisodia SS, Price DL. Transgenic models of neurodegenerative diseases. *Current opinion in neurobiology*. 1996 Oct 1;6(5):651-60.
7. World Health Organization. Neurological disorders: public health challenges. World Health Organization; 2006.
8. Mathur G, Pareek A, Dave V, Nain S, Sharma S. Dementia: at a glance. *International Journal of Advanced Research in Pharmaceutical & Bio Sciences*. 2013 Jul 1;2(4):1-0.
9. Roach AP, West S. Identification trainers for the future: developing the next generation of expert naturalists at the Angela Marmont Centre for UK Biodiversity. *J. Nat. Sci. Coll*. 2018;5:78-88.

10. Cousin NC, Parker A, Sidler K. The Use of Fluorescence in Genetically Modified Animals.
11. Harvey EN. Evolution and bioluminescence. *The Quarterly review of biology*. 1956 Dec 1;31(4):270-87.
12. Jeng ML. Biofluorescence in terrestrial animals, with emphasis on fireflies: A review and field observation. *Bioluminescence-Analytical Applications and Basic Biology*. 2019 Aug 28.
13. Su KH, Mante PK, Xavier-Elsas P. Model organisms in experimental pharmacology and drug discovery 2023: rodent, worm and zebrafish models. *Frontiers in Pharmacology*. 2024 Aug 8;15:1462972.
14. Watts JC, Giles K, Grillo SK, Lemus A, DeArmond SJ, Prusiner SB. Bioluminescence imaging of A β deposition in bigenic mouse models of Alzheimer's disease. *Proceedings of the National Academy of Sciences*. 2011 Feb 8;108(6):2528-33.
15. Bernis ME, Tamgüney G. Bioluminescence Imaging of Neuroinflammation in a Mouse Model of Parkinson's Disease. *InBioluminescent Imaging: Methods and Protocols* 2019 Nov 13 (pp. 147-159). New York, NY: Springer US.
16. Yang J, Ding W, Zhu B, Zhen S, Kuang S, Yang J, Zhang C, Wang P, Yang F, Yang L, Yin W. Bioluminescence Imaging with Functional Amyloid Reservoirs in Alzheimer's Disease Models. *Analytical Chemistry*. 2023 Sep 15;95(38):14261-70.
17. Hochgräfe K, Mandelkow EM. Making the brain glow: in vivo bioluminescence imaging to study neurodegeneration. *Molecular neurobiology*. 2013 Jun;47(3):868-82.
18. Calvo-Rodriguez M, Hou SS, Snyder AC, Dujardin S, Shirani H, Nilsson KP, Bacskai BJ. In vivo detection of tau fibrils and amyloid β aggregates with luminescent conjugated oligothiophenes and multiphoton microscopy. *Acta neuropathologica communications*. 2019 Nov 8;7(1):171.
19. Tokuraku K, Marquardt M, Ikezu T. Real-time imaging and quantification of amyloid- β peptide aggregates by novel quantum-dot nanoprobes. *PloS one*. 2009 Dec 30;4(12):e8492.
20. Yang J, Ding W, Zhu B, Zhen S, Kuang S, Yang J, Zhang C, Wang P, Yang F, Yang L, Yin W. Bioluminescence Imaging with Functional Amyloid Reservoirs in Alzheimer's Disease Models. *Analytical Chemistry*. 2023 Sep 15;95(38):14261-70.
21. Luo J, Ho P, Steinman L, Wyss-Coray T. Bioluminescence in vivo imaging of autoimmune encephalomyelitis

- predicts disease. *Journal of neuroinflammation*. 2008 Feb 1;5(1):6.
22. Watts JC, Giles K, Grillo SK, Lemus A, DeArmond SJ, Prusiner SB. Bioluminescence imaging of A β deposition in bigenic mouse models of Alzheimer's disease. *Proceedings of the National Academy of Sciences*. 2011 Feb 8;108(6):2528-33.
 23. Fricke IB, Schelhaas S, Zinnhardt B, Viel T, Hermann S, Couillard-Després S, Jacobs AH. In vivo bioluminescence imaging of neurogenesis—the role of the blood brain barrier in an experimental model of Parkinson's disease. *European Journal of Neuroscience*. 2017 Apr;45(7):975-86.
 24. Naumann EA, Kampff AR, Prober DA, Schier AF, Engert F. Monitoring neural activity with bioluminescence during natural behavior. *Nature neuroscience*. 2010 Apr;13(4):513-20.
 25. Szentirmai O, Baker CH, Lin N, Szucs S, Takahashi M, Kiryu S, Kung AL, Mulligan RC, Carter BS. Noninvasive bioluminescence imaging of luciferase expressing intracranial U87 xenografts: correlation with magnetic resonance imaging determined tumor volume and longitudinal use in assessing tumor growth and antiangiogenic treatment effect. *Neurosurgery*. 2006 Feb 1;58(2):365-72.
 26. Dunn-Meynell AA, Dowling P, Marchese M, Rodriguez E, Blumberg B, Choi YB, Gainth D, Lu W. In vivo bioluminescence imaging used to monitor disease activity and therapeutic response in a mouse model of tauopathy. *Frontiers in Aging Neuroscience*. 2019 Sep 12; 11:252.
 27. Manni I, Di Rocco G, Fusco S, Leone L, Barbati SA, Carapella CM, Grassi C, Piaggio G, Toietta G. Monitoring the response of hyperbilirubinemia in the mouse brain by in vivo bioluminescence imaging. *International Journal of Molecular Sciences*. 2016 Dec 28;18(1):50.
 28. Im HJ, Hwang DW, Lee HK, Jang J, Lee S, Youn H, Jin Y, Kim SU, Kim EE, Kim YS, Lee DS. In vivo visualization and monitoring of viable neural stem cells using noninvasive bioluminescence imaging in the 6-hydroxydopamine-induced mouse model of Parkinson disease. *Molecular Imaging*. 2013 Jun 1;12(4):7290-2012.
 29. Basu S, Adams L, Guhathakurta S, Kim YS. A novel tool for monitoring endogenous alpha-synuclein transcription by NanoLuciferase tag insertion at the 3' end using CRISPR-Cas9 genome editing technique. *Scientific reports*. 2017 Apr 4;7(1):45883

30. Gandy A, Maussion G, Al-Habyan S, Nicouleau M, You Z, Chen CX, Abdian N, Aprahamian N, Krahn AI, Larocque L, Durcan TM. An Inducible Luminescent System to Explore Parkinson's Disease-Associated Genes. *International Journal of Molecular Sciences*. 2024 Aug 31;25(17):9493.
31. Paquet D, Bhat R, Sydow A, Mandelkow EM, Berg S, Hellberg S, Fäلتing J, Distel M, Köster RW, Schmid B, Haass C. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *The Journal of clinical investigation*. 2009 May 1;119(5):1382-95.
32. Yang KD, Belyaeva A, Venkatachalapathy S, Damodaran K, Katcoff A, Radhakrishnan A, Shivashankar GV, Uhler C. Multi-domain translation between single-cell imaging and sequencing data using autoencoders. *Nature communications*. 2021 Jan 4;12(1):31.
33. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J. The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature*. 2016 Sep 1;537(7618):50-6.
34. Regan, T. (1983). *The Case for Animal Rights*. University of California Press.
35. Russell, W. M. S., & Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. Methuen.
36. Levy, S. (2003). GloFish: Night lights for your aquarium. *The Scientist*, 17(24), 23.
37. Zhou, W., De Iaco, A., & Schorpp, M. (2016). Advances in genetic engineering of model organisms: Transgenesis and gene targeting. *Developmental Biology*, 416(1), 67–76.
38. Snow, A. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M., & Wolfenbarger, L. L. (2005). Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Applications*, 15(2), 377–404.
39. Carbone, L. (2004). *What Animals Want: Expertise and Advocacy in Laboratory Animal Welfare Policy*. Oxford University Press.
40. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, Macleod MR. Can animal models of disease reliably inform human studies?. *PLoS medicine*. 2010 Mar 30;7(3):e1000245.

41. Perrin S. Preclinical research: Make mouse studies work. *Nature*. 2014 Mar 27;507(7493):423-5.
42. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013 Feb 15;339(6121):819-23.
43. Moen E, Bannon D, Kudo T, Graf W, Covert M, Van Valen D. Deep learning for cellular image analysis. *Nature methods*. 2019 Dec;16(12):1233-46.
44. Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AK, Pak S. Structural and molecular interrogation of intact biological systems. *Nature*. 2013 May 16;497(7449):332-7.