

Chromoprotein and eumelanin: a synthetic pathway for the production of safer hair dye*

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*The widespread use of conventional hair dyes has raised substantial concerns regarding their safety and long-term effects. Many commercial formulations include harmful chemicals such as ammonia, hydrogen peroxide, and p-phenylenediamine, which are associated with allergic reactions, skin irritation, and potential carcinogenicity risks. Our design offers a novel and safer alternative by harnessing melanin—the natural pigment responsible for hair color— and chromoproteins, which are vividly colored proteins containing prosthetic groups. These pigments are biosynthesized via genetically engineered *Escherichia coli*. Chromoproteins are proteins that contain a colored prosthetic group, giving them a vivid color to enhance the color variety of *E. coli*. Our design intends to utilize a previously engineered strain of *E. coli*. By extracting the plasmid from *E. coli*, we can utilize this innovation to create the desired color by expressing these proteins. Once extracted, the plasmid is cut open, allowing us to insert a melanin-producing DNA sequence. This new modification allows for the creation of numerous new hues that can then be implemented into yeast to be prepared for a final extraction.*

Keywords: Eumelanin, melanin, *E. coli*, plasmid, pigmented yeast, hair dye, synthetic hair dye, chromoprotein



Conventional hair dyes have raised widespread concern among consumers, particularly regarding their safety. Specifically, 68-80% of at-home hair dye users in the United States report safety-related worries, with growing concern for users who have faced irritation due to the contents of the product (Duzick, 2021). Even among individuals who have never dyed their hair, about one-third of middle-aged adults express apprehension about at-home hair dyes, concerns that often arise as their hair begins to gray (Duzick, 2021). Hair color begins to gray as people age due to the aging of melanocytes, the cells that produce melanin, alongside the person (Weintrob,

2022). As the melanocytes in the hair follicles die, the pigment ceases to create color, leaving it gray as seen in Figure 1



Figure 1. The effect of melanin on hair follicles.

* The authors were mentored by Beth Pethel from Western Reserve Academy and Joe Switzer from BioBuilder Educational Foundation. Please direct correspondence to: {Email Addresses}. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2025. It is distributed under the terms of the Creative Commons Attribution License, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

(Weintrob, 2022).

The difference is shown between two fair follicles, one of which is pigmented by the production of melanin and the other of which has turned gray due to the lack of produced melanocytes (Windsor, 2020).

Although all humans age with time, a rare percentage of individuals experience more severe hair graying in the early stages of their midlife.

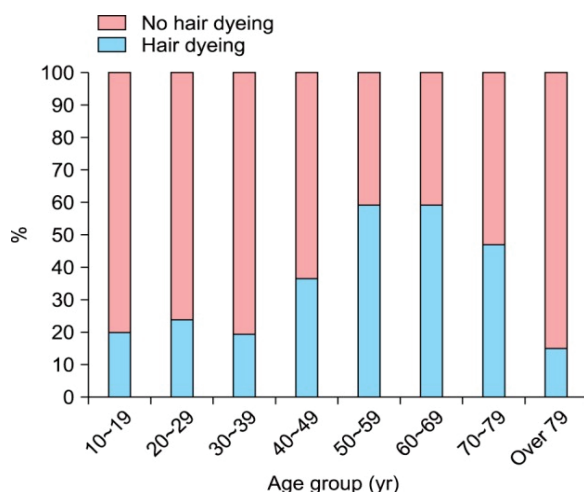


Figure 2. The proportion of individuals with dyed hair by age group.

There is a greater percentage of individuals dying their hair at ages 40-79; in this study, the researchers investigate the use of hair dye among Koreans (Jo, et al., 2013).

Over seventy million middle-aged adults face the problem of graying hair, with 6-23% of those in their fifties (Reed, 2023). Due to the negative connotations associated with graying hair, such as the perception of impending old age, adults often favor masking it through the use of hair dye, as shown in Figure 2. Despite many attempts to cure or slow the process of graying hair, most existing practical solutions, such as graphene-based sheets or gold particles, have unfavorable side effects. Graphene-based sheets apply graphene onto hair (Luo et al., 2018), and gold particles are synthesized to form hair color (Bioparticles.com, 2021). The most notable adverse effects of these products include allergic reactions, skin irritation, hair loss, and potential cancer risks (Hair Dyes, 2022). The toxicity of many hair dyes can lead to skin diseases, such as discoid

lupus erythematosus (DLE) or systemic lupus erythematosus (SLE). Hair dyes have been proven to possibly even risk the chance of fatal outcomes from pneumothorax or acute kidney injury (AKI) (He et al., n.d., 2022). The inefficiencies of existing solutions may lead consumers to seek alternatives; unfortunately, however, there are no products currently providing long-term and safe solutions (Narayana, 2013).

Due to a large percentage of the global population using hair dyes, it has become evident that many of these dyes cause widespread harm to consumers despite their plentiful benefits (Hair Dyes, 2022). Currently, there are very few alternative solutions to hair dyeing methods, suggesting that a synthetically engineered solution presents an opportunity to address this ongoing issue.

Conventional hair dye is known to have detrimental effects, including structural damage to the hair shaft (He et al., 2023) and potential carcinogenic consequences following its use (Zhang et al., 2020). Therefore, current research aims to address the issues of hair dye safety and sustainability in graying hair. A key example of research on coloring graying hair involves using nanoreactors to target the hair fiber. (Haveli et al., 2012). Nanoreactors are minuscule reaction vessels that can catalyze and regulate chemical reactions at the nanoscale level (Goksu et al., 2023). By treating hair fibers with nanoreactors, the group discovered that gold nanoparticles (AgNP) synthesized in human hair could produce a deep brown pigment in white hair (Haveli et al., 2012).

In hair, gold nanoparticles require extensive time, from seven hours up to sixteen days, to develop color (Haveli et al., 2012). Although the innovation provided a long-lasting, water-resistant color, their experiment had limited color options, as synthesizing gold nanoparticles in an alkaline solution would only shift the hair's gray or white color to a deep black. Another downside was the costly procedure (Haveli et al., 2012). Due to its expensive process and inflexibility their experiment will unlikely make it to a salon in the long run.

Dyeing gray hair similarly raises concerns regarding the durability and safety

of repeated hair dye applications; over time, gray hairs reappear as part of the natural hair growth cycle, irrespective of the dyeing method used. A group of researchers developed a graphene-based hair dye using graphene sheets that address concerns regarding safety and performance (Luo et al., 2018). Since graphene is an allotrope (chemical elements that can exist in two or more forms) of carbon positioned in a hexagonal lattice to assemble single-atom-thick, two-dimensional sheets (Elabd & Coskun, 2018), its thermal and electrical abilities have made it a primary subject of study for durable hair dye. Graphene hair dyes do not contain toxic molecular components, resolving the safety issue, and their durability has reached the performance of permanent hair dyes (Luo et al., 2018). Although the researchers use a cheaper rendition of graphene, graphene oxide (Luo et al., 2018), the process remains costly from a large-scale perspective, where cost-performance factors diminish the viability of graphene as an alternative to permanent hair dyes. Despite the prevalence of hair dye in masking gray hair, its usage faces challenges that innovative experiments aim to address. These promising innovations are hindered by high production costs, which limit the scalability of graphene-based dyes as practical alternatives.

Given these limitations, biologically engineered solutions may offer a more sustainable and cost-effective path forward. Our design produces a melanin and pigment-based hair dye utilizing *Escherichia coli* (*E. coli*) as a chassis. If we can successfully transform the human melanin-producing pathway into *E. coli*, we will have the opportunity to extract the pigments produced by the organism. We aim to engineer *E. coli* with the melanin biosynthesis pathway, enabling the microbial production and extraction of natural hair pigments.

This eumelanin biosynthesis pathway stems from Tyrosine and undergoes synthesis to produce eumelanin. The melanin production pathway produces pigments such as black (from eumelanin), brown (also from eumelanin), and yellow (from pheomelanin) (Adhikari, et al., 2018).

Using the process shown in Figure 3, we integrated melanin into *E. coli* to change the

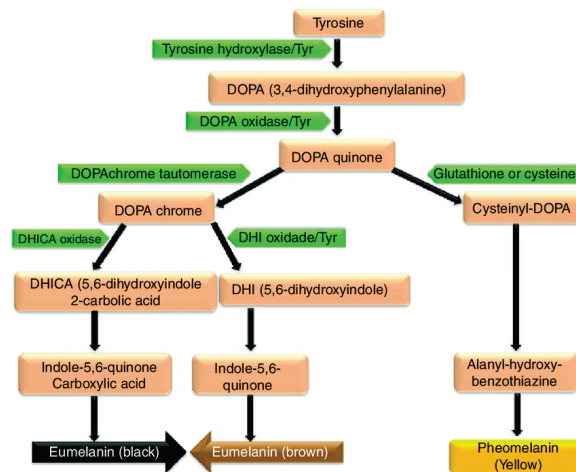


Figure 3. Melanin production pathway from Tyrosine.

shade of the dye. The new *E. coli* could provide a natural color production without risk. This process introduces the possibility of a natural hair dye whose main base comes from a natural organism. However, instead of using the entirety of *E. coli*, we introduce the biological pathway to create melanin and extract the pigment produced. By combining the *E. coli*'s plasmid with pigmented yeast, we can adjust pigmentation to the preferred color using our arabinose-inducible promoter. The arabinose acts as an on-and-off switch for allowing the induction of melanin into the plasmid, which, depending on how much or how little melanin is produced, the plasmid will have a multitude of hues to change to, as seen in Figure 3. They found that this pathway could create melanin for skin disorders. This study, therefore, indicates that using this pathway is a potential alternative method for producing pigments for use in hair dyes.

Systems level

Our design utilizes a plasmid from modified *Escherichia coli* with the gene coding for black eumelanin implemented into it; then, the plasmid will be added to pigmented yeast to alter the color before extracting it. This solution solves the safety concerns associated with common hair dyes, as it eliminates the need for cytotoxic reagents, offering a biocompatible and user-friendly alternative

to conventional hair dye. The methodology proposed in the current study is based on that by Cutiful; however, it includes the implementation of pigmented yeast to alter the color hue and allow for the production of customizable colors. This idea provoked the creation of a hair dye that achieved all of the safety solutions developed through the previous study by adding melanin and yeast as the host organism. Implementing these two changes allows the alteration of the color and shade of the hair dye.

Device level

Our design employs a three-step process that gradually incorporates additional genes, resulting in a low-risk synthetic hair dye that can later be modified using a promoter sequence; those steps are illustrated in Figure 4.

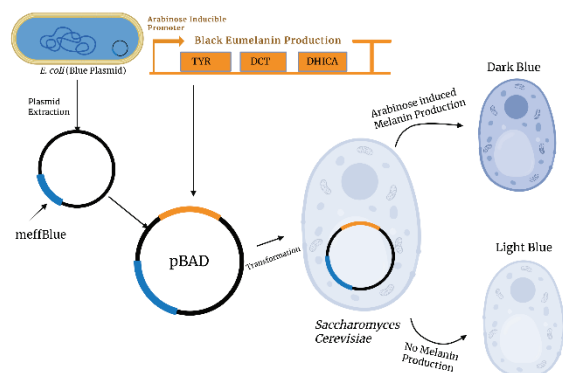


Figure 4. Color production process from *E. coli* to Arabinose-induced melanin production inside yeast.

The pBADplasmid containing the meffBlue gene, which is the chromoprotein responsible for giving the plasmid its blue color, is extracted from *E. coli* and ligated into the pBAD vector containing an arabinose-inducible promoter driving the expression of genes involved in black eumelanin biosynthesis: Tyrosine hydroxylase (TYR), DOPAchrome tautomerase (DCT), and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). A chromoprotein such as the meffBlue gene is a protein that displays visible color. The recombinant pBAD

plasmid is then transformed into *S. cerevisiae*. In the presence of arabinose, the promoter is activated, leading to the expression of melanin biosynthetic enzymes and subsequent melanin production, resulting in a dark blue yeast phenotype. In the absence of arabinose, no melanin is produced, and the yeast remains light blue.

The first step is the extraction of pBAD, a plasmid containing the meffBlue gene from *E. coli*, specifically the *E. coli* strain DH5a altered by Cutiful, a team in the United Kingdom. Extracting the plasmid from this altered *E. coli* creates a new possible contributor in the creation of a color enzyme, which is the foundation for the color of the hair dye.

In the following step, we introduce the black eumelanin pathway into the existing *E. coli* plasmid; this design offers flexibility in hair color and takes advantage of the natural color of melanin. The genes in this pathway include TYR (Tyrosine hydroxylase), DCT (DOPAchrome tautomerase), and DHICA oxidase (5,6-dihydroxyindole-2-carboxylic acid), ultimately leading to the production of eumelanin controlled through pBAD, the promoter of these proteins. Incorporating a promoter into the pigment-producing system enables adjustable gene expression, allowing for a wide range of shade variations and a more diverse color palette, as shown in Figure 5. A diverse color palette can be achieved through the substitution of the original *E. coli* that holds the corresponding chromoprotein.

One of the primary objectives of our design is to accommodate a wide range of different hues. The aim is for there to be no



Figure 5. Producible Color Palettes.

limit to the color that can be produced, with the only limiting factor being the color of the plasmid within the *E. coli*, but still allowing for a large range. This eliminates the need to use a different product that does not provide the same benefits or to acquire a more expensive product.

The third and final step involves introducing our modified plasmid into a compatible and safe yeast, *Saccharomyces cerevisiae*. As the carrier of our plasmid, it will produce large quantities of the chosen color. The color of the pigment is created through a two-part process where melanin production occurs from the plasmid, which is engineered to produce high-yield output. This final product would be processed by extracting the pigment from the yeast. After purifying the resulting pigment, it can be added into a hair dye solution as its Pigment.

Parts level

The design of the *E. coli* hair dye system uses genetically modified *E. coli* and pigmented *S. cerevisiae* to create a safe and sustainable alternative to conventional hair dye. Initially, the blue chromoprotein gene (meffBlue) containing plasmid is extracted from *E. coli*. Subsequently, the plasmid is modified by implementing an arabinose-inducible promoter to include genes from the black eumelanin production process. The addition of the *Tyrosine hydroxylase* (TYR), *DOPAchrome tautomerase* (DCT), and *DHICA* black eumelanin genes enables regulated melanin production. The melanin production sequence is shown in greater detail in image 6. Following the addition of the black eumelanin genes, the plasmid is introduced into the *S. cerevisiae* yeast through transformation. The plasmid enables the synthesis of tyrosine into melanin production once inside the yeast, where the color of the yeast cells will confirm the impact of the arabinose on the plasmid, since arabinose catabolism in *E. coli* is a pattern that aids genetic regulation. Ultimately, the remaining pigment is extracted from the yeast to manufacture the final hair dye product.

This linear flow diagram depicts the creation of black eumelanin starting from

Tyrosine. The diagram continually adds

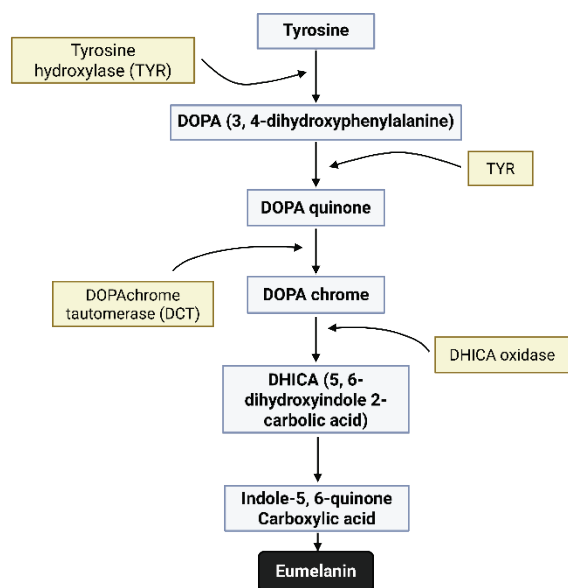


Figure 6. Eumelanin production sequence.

genes throughout its process, showing their placement inside it. The eumelanin is vital in the design as it is the primary way the hue will be changed when preparing to turn into a final product.

Safety

E. coli is known to be a remarkably safe bacterium to work with. Many scientists and researchers have worked with *E. coli* for a long time; at this point, it still sits at a BioSafety Level 1 (BSL - 1), depending on the strain used. In our design, we utilize BL21, which is a commonly used strain that poses minimal risk to humans. If exposed to certain strains of *E. coli*, a worker can contract typical illnesses such as a urinary tract infection (UTI), pneumonia, bacteremia, meningitis, and abdominal infections, among others (Mueller & Tainter, 2023). Working with this organism requires safety precautions, such as wearing safety glasses, lab coats, gloves, a mask, and always making sure your skin is not exposed to the *E. coli*. With this in mind, we find it essential that consumers recognize that the *E. coli* strain used in our product not only was found to be harmless as it is already genetically modified from previous work, but it also sits at only a

BSL - 1, making working with it completely safe and its use in the design safe for consumerism.

MeffBlue is a chromoprotein commonly engineered into *E. coli*. Chromoproteins are not a danger to the human body and, in many cases, have even been used in various ways to cure the body of an illness, making the risk of using *MeffBlue* slim to none (*Regulators of Growth*, 2003).

Saccharomyces cerevisiae, more commonly known as baker's yeast, is a non-pathogenic yeast typically used for baking bread or brewing beer. This yeast poses no danger to the human body in the same way it is commonly consumed. It should still be noted that the baker's yeast will not be a part of the final product, as it is only used as a factory to produce the final pigment for the hair dye.

To safely test our product, we would use standard hair dye testing practices found to be utilized throughout the hair products industry. The method is to apply a small amount of the dye to a small skin patch, generally behind the ear, and leave it for up to 48 hours. If no signs of burning, irritation, or other indications of allergic reactions occur, it is safe to be used on the hair (He et al., 2022).

Discussions

The melanin-based hair dye which is engineered through *E. coli*, presents several promising advantages over conventional hair dyes. Conventional hair dyes contain ammonia, hydrogen peroxide, and p-phenylenediamine. However, a natural pigment-producing pathway can significantly reduce the risk of harmful side effects such as irritation, toxicity, and allergic reactions commonly associated with traditional chemical and nanoparticle-based dyes. Therefore, we address safety and versatility issues by integrating melanin with the *E. coli*-derived palette while expanding the range of achievable colors. Combining plasmids and melanin-producing DNA sequences represents a customizable approach to pigment production, allowing users to determine the hue and saturation of their hair color. However, we must

acknowledge a few limitations of our project. Since the study remains at the design stage, we have not yet tested our system experimentally. The pigment extraction efficiency and stability under varying environmental conditions remain unknown and warrant further investigation. Moreover, scaling the unconventional hair dye process for commercial use poses challenges, particularly in maintaining consistent gene expression and optimising bioreactor conditions. Future efforts should prioritize increasing pigment yield and developing cost-effective strategies for large-scale production. Addressing these limitations will be essential to refining our design into a safe, accessible, and sustainable alternative for real-world hair dye applications.

Next steps

A future implication of creating our hair dye is the experimentation and classification of colors produced through melanin. The effectiveness of eumelanin in the process is crucial to the effectiveness of the dye as an effective hair dye, not just a safe one. The quantity of melanin produced through the eumelanin production sequence would alter the overall color of the dye relative to the addition of Arabinose. The dye output would enable understanding the eumelanin-to-color ratio required for optimal coloration. A fundamental change that could further assist this project would be replacing *Saccharomyces cerevisiae*, the host yeast. Other host organisms that could potentially work as a replacement would be classified as BSL-1, easily accessible, and adaptable to the already constructed plasmid. Experimentation with the host cell aspect of our design could result in dramatic or minimal changes to the creation of chromoproteins. This change could be beneficial in increasing the output of chromoproteins, leading to a more expressive and productive outcome, thereby elevating both design and productivity. Experimentation in replacing the host cell would be an accomplishable task for the future of hair dye.

Author contributions

T. J. devised the concept, designed the study, and created all the images and videos. M. W. wrote the abstract, tracked research progress, and reviewed all written works. A. L. wrote the Next Steps and conducted thorough research through fact-checking. M. W. and A. L. collaboratively wrote the Systems, Device, and Parts Level sections.

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