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Dear Reader,

We are pleased to introduce the Stanford Undergraduate Research Journal's twentieth volume. After a COVID-induced hiatus, SURJ is back with a full staff of dedicated editors who have spent that last year working to rebuild the website, combing through the submissions archive, and re-establishing the journal's club status. After months of hard work, we are thrilled to present our first publication in two years.

With the rising spread of misinformation, it is imperative that strong, peer-reviewed research is not ignored. The times we live in are wrought with global unrest and a general trend toward individualism in a tech-driven world. Wars in Eastern Europe and the Middle East remain ongoing, humanitarian aid is being stripped away despite international food shortages, xenophobia is on the rise, and medical expertise is ignored in favor of convenience. We feel that now, perhaps more than ever, diverse voices must be uplifted, and the status quo challenged.

Our articles cover topics from the intricacies of the war between Russia and Ukraine to the acceleration of protein folding in vivo. Authors in the Humanities & Social Sciences section investigate the appearances of English words in French rap music, analyze the role of patriarchy in the redemption arc of *Disgrace*, and consider how photography changes the viewing experience of Earthworks art installations. In the Natural Sciences & Engineering section, authors examine the toxicity of engineered nanoparticles on living cells; develop improvements to emerging technologies for water purification; and illuminate the benefits of genetically modified plants for agricultural productivity, nutritional enhancement, and pesticide reduction.

Since its founding in 2001, SURJ has produced intellectually vibrant collections of outstanding undergraduate research to celebrate students' accomplishments, share their discoveries, and promote an investigative spirit among undergraduates. SURJ's mission is to encourage, recognize, and reward intellectual activity outside the classroom, and we hope that through this publication, we can continue to foster undergraduates' pursuit of creativity and knowledge.

We would like to thank our journal's advisor and professor emeritus of the Stanford Graduate School of Education, Dr. John Willinsky, for his guidance and support. Without him, it would not have been possible to get the journal up and running again. We would also like to thank Mr. Wilson Wang, the Assistant Director of Data Science at the GSE for his help behind the scenes with Open Journal Systems (OJS). Finally, we would like to express gratitude for our Review Board and their dedication to providing detailed feedback on every article.

Congratulations to all of the talented authors who were selected for publication. We were thoroughly impressed and inspired by this work and hope that you will be too!

Sincerely,

Aaron Adam ('25) & Natalie Rodriguez ('25)

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# “Trop clean pour toi”: Investigating the Diverse Dynamics of English Language Borrowing in French Rap Music

MAX JARDETZKY

STANFORD UNIVERSITY

This paper evaluates English borrowing in French rap music in the contexts of mainland France and French-speaking Canada. English borrowing exists against a backdrop of systemic linguistic purism, such as a 1994 French law requiring that at least 40 percent of songs played on air be in French, “[stemming] the encroachment of English into French culture” [5]. Literature in sociolinguistics has evaluated non-standard language use in French rap more broadly but lacks fine-grained analysis of English borrowing. The author categorizes and contextualizes computationally extracted English borrowings through a novel lexical subcategorization framework that anchors these borrowing practices in discrete sociocultural contexts. The study finds that French rappers strategically integrate English elements into their songs as a means of resistance and identity assertion. In France, where linguistic hegemony prevails, English borrowings are syntactically limited but semantically diverse. Constructions range from noun phrases to reinterpretations of idioms, with occasional code-switching. On the other hand, in a case study of Canadian French rap, *Franglais* emerges as fluid code switching that reflects the region’s mature linguistic heterogeneity. The author argues that by challenging linguistic purism with non-standard language practices, rappers are asserting translinguistic identities and amplifying marginalized communities. Overall, the findings underscore the importance of empowering non-standard language use in music, as it fosters cultural representation and linguistic evolution.

**Keywords:** borrowing, linguistic purism, English, French, French rap, sociolinguistics, computational linguistics, linguistics, non-standard language, France, Canada

## Introduction: A Rap Practice That Transcends Standardized Language

French rap music is a colorful genre that has evolved greatly in the last few decades. Since its inception in the 1990s, its artists have aimed to “reassess French national history and national self-perception” [1]. This sentiment persists in today’s French rap, which is now undeniably centered around the lived experience in *les banlieues*, an almost untranslatable French word referring to lower-middle class communities within suburban housing installations. These communities are highly linguistically and culturally diverse, and many inhabitants are second-generation immigrants. There, rap music has become an essential means of identity performance, which is often mediated by non-standard French. Non-standard French includes the language game of *verlan* (systematic word inversion), various other forms of French slang, and foreign language borrowing or code-switching. For an illustrative example, within the popular song CANADA by French rapper 1PLIKÉ140, I located five major instances of non-standard French occurring within ten seconds:

<i>C'est les quartiers d'France, ça s'la fout<sup>1</sup> pour un 'ça vient d'où?' (Pour un 'ça vient d'où?')</i>	These are the French districts, <u>fighting</u> upon a ‘where’d you come from?’
<i>What's you gonna do<sup>2</sup> si mes gars<sup>3</sup> prennent ta Canada</i>	<u>What's you gonna do</u> if <u>my guys</u> take your Canada [referring to the brand Canada Goose]
<i>Location toute l'année mais t'as <u>walou</u><sup>3</sup>, en vrai, t'as <u>nada</u><sup>4</sup> [2]</i>	Rental all year but you’ve <u>nothing</u> , you’ve <u>nothing</u> [chart rank #25, my translation]

1. French slang
2. English code-switching
3. Maghrebi Arabic borrowing
4. Spanish borrowing

Notice how within these lyrics, 1PLIKÉ140 seamlessly weaves non-standard French from diverse sources, constructing a speech act

with remarkable rhetorical variety. My research solely focuses on English language borrowings in French rap, which occur at a high frequency.

### Background: Institutional Linguistic Purism in France and Eastern Canada

Since sociocultural context influences these rappers' relationships with language, I will frame my analysis in terms of two particular genre spheres where French rap is most prominent: those of mainland France and eastern, French-speaking Canada. The dominant culture in both genre spheres exert high degrees of linguistic purism, or social pressure to adhere to what is deemed pure French. Non-standard French thus entails any deviation from the prescribed "grammar, syntax, vocabulary and pronunciation" [3, p. 282]. The act of English borrowing particularly upsets French language authorities. In 2013, *L'Académie Française*, the de facto ruling body of European French, published a manifesto called *À la reconquête de la langue française* (Reconquering the French language). Within it, its Perpetual Secretary Hélène Carrère d'Encausse wrote, "*La langue française est ... menacée ... par la langue anglaise qui insidieusement la dévore de l'intérieur*" ("The French language is menaced ... by the English language, which is insidiously devouring it from the inside") [4, para. 7]. Rich in pathos, the personifying language gives a grotesque mental image. Carrère d'Encausse paints the desperate plight of standard French, urging the public to dedicate to preserving it.

These sentiments of linguistic purism boil down into the music industry, challenging the opportunities and perceptions of French rappers within greater society. In France, a 1994 law requires that 40 percent of all songs played on air are in the French language, in part to "stem the encroachment of English into French culture" [5]. French radio stations protested an amendment to the law with a day-long boycott in 2015, stating that the record industry was using language preservation as a thin veil to further its economic interests. I believe that while this law most directly concerns foreign music imports, it establishes a dangerous precedent at home in the form of a dividing line on what constitutes sufficient French-language content. It would also work to exclude "homegrown artists increasingly choosing to sing in English to boost their commercial appeal," as France 24 also states [5]. Later, I will establish that deviations from standard French reflect more than just commercial opportunity, rather an ongoing process of identity negotiation.

On the subject of dividing lines, music industry authorities in eastern Canada attempt to limit the appearance of English in new works, while *Franglais*, the fluid mixing of French and English, so well defines this region's language identity. Furthermore, according to Canadian social anthropologist Bob White, the practice of *Franglais* "may be emerging as an aesthetic marker for the sub-genre of Québécois rap" [6, p. 963]. Under a 30-year-old rule in Québec, a certain \$18,000 government-funded grant for emerging musicians can only be awarded if the project's lyrical content is at least 70 percent French. In 2016, the prominent hip-hop band Dead Obies lost out on the grant when their album ended up being 55 percent French and 45 percent English [7]. I tasked myself with how English borrowing plays into this sociolinguistic tension, in which French rappers so strongly identify with non-standard language use and position themselves relative to a dominant culture that wields purist language ideologies. I discovered that by borrowing English words, French rappers are establishing translinguistic identities and

challenging narratives of linguistic purism. I seek to demonstrate this by locating and categorizing English borrowings and illuminating their lyrical and social contexts.

But first, I must showcase scholars' existing approaches and establish a research gap around the nature and significance of English borrowing and code-switching. Prior literature has failed to reflect the depth of the practice, motivating my computational primary source research which located English borrowings on recent Top 100 rap charts in France and Canada. This primary data helped me construct a novel lexical subcategorization framework for English borrowings on the mainland French rap charts, which I will use to illuminate the practice within that genre sphere. In Canada, limited song data necessitates a case study on how one Québécois rapper, FouKi, employs *Franglais* for his own identity construction. After my theoretical treatment of English borrowing in mainland French rap and *Franglais* in French Canadian rap respectively, I will move to situate these borrowings in their sociolinguistic contexts and argue an emergent, unifying rhetorical significance.

### Literature Review: Establishing the Need for More Refined Categorization

Before I enter my analysis, I would like to position myself relative to the work of several researchers who investigate English as it appears in French rap music. European sociolinguist Martin Verbeke performs quantitative lexicographic analysis on non-standard language frequency in French rap. He has considered categories such as rappers' associated nationalities, cities, Parisian suburbs, decades, and subgenres. While Verbeke's methodology of lexicographic analysis can produce exact percentages regarding the frequencies of non-standard language categories within a lyrical corpus, it is too broad to offer nuance to the practice of English borrowing. However, this is not to say that subcategorization of English word appearances has never been attempted. French literature scholar Skye Paine provides a novel framework by dividing English borrowings into the categories of "the useful and the ornamental," meaning essential and cosmetic [8, p. 63]. Through my primary research, I discovered that these categories proved utterly insufficient to the present practice of English borrowing, so this is where I identify the gap in existing literature. I will adapt the practice of lexicographic analysis and supplement it with subcategorizations, which I believe more accurately reflect the dynamic practice of English borrowing in mainland French rap music.

Since I equally value the social implications of the practice, I consulted research with more qualitative, socially situated analyses, beginning with that of French-specialized linguist Samira Hassa. She examines the social motivations behind English and Arabic borrowings and *verlan* in French rap. However, I found that Hassa reductively characterizes non-standard language occurrences as vectors for discussions of violence, drug culture, and other *banlieue* delinquency [9, p. 58]. In my own corpus, I observed borrowings with stylistic purposes beyond those that Hassa enumerates, which further reflects the research gap. In his genre-based paper, Verbeke adapts symbolic classifications for American rap music videos from scholars Conrad et al. to create the following qualitative framework for analyzing French rap music videos: "materialism..., misogyny..., violence..., political awareness..., expression of culture..., disaffection with mainstream society..., [and] group unity" [10, p. 55]. I appreciate that this qualitative categorization framework is much less socially isolated than Paine's. However, none like it has yet been



applied to rap lyrics, which is exactly what I aim to do with my analysis.

Researchers in the domains of sociolinguistics and cultural anthropology have aimed to deconstruct the language practice of Franglais in French rap, categorizing English influences. White builds upon Myers-Scotton's Matrix Language-Frame model of code-switching, which establishes a dominant (matrix) language that provides syntax and a subordinate (embedded) language which provides content morphemes [6]. His basic unit of analysis is the complementizer projection, referring to both the underlying syntactic structure of an instance of borrowing and the overlaid morphemes that provide content or establish relations. White chooses the 17-song album *Montréal \$ud* by Dead Obies from 2013 as a representative corpus and identifies nearly 2,000 complementizer projections within it, concluding with a high-level interpretation of the syntactic dynamics of the Franglais that appears. He argues that existing anxieties around how Franglais challenges the future of French are unfounded, as English code-switching seems to exist primarily for cosmetic aims and for these rappers to establish themselves as "creative agents or as linguistic innovators" [6, p. 964]. The particularities of Canadian Franglais necessitate this more generalized frame of analysis, as it often involves both French borrowings into English and English borrowings into French. For mainland French rap where French structure dominates, the Matrix Language Frame model reduces to a single case, which would demand a more subdivided framework. While White's methodology is attentive to the depth of Franglais, his lyrical corpus is a single nine-year-old rap album, which is outdated and too narrowly scoped. For both genre spheres, I will analyze English borrowings by applying subcategorization frameworks upon currently relevant lyrical data from my primary research.

## Methodology: Playing the Computational Card.

The lack of up-to-date and transparent analysis on the intricacies of English language borrowing in French language rap music motivated my research methodology as follows. I wanted to conduct an impartial inquiry on how English appears within this genre, so I began with the Shazam Top 100 rap chart in France from the week of April 8<sup>th</sup>–15<sup>th</sup>, 2022 to establish my raw dataset. I added song lyrics to it and removed non-French songs as they did not reflect my narrow research scope. I then wrote a C++ program to match English word occurrences from a 370,000-word lexicon, generating a large text file of English borrowings labeled by artist, title, and chart position. In the file, each occurrence of English borrowing was placed in its lyrical context. Occurrences were categorized by word, automatically contrasting how the same word can appear differently across popular songs. I manually filtered out thousands of false positives due to French-English cognates, according to dictionary presence and my own knowledge.

By returning to my original lyrical dataset and cross-referencing these contextualized matches, I constructed a 70-page document with the lyrics of 52 relevant target songs, bolding lines containing English borrowings as previously identified by my algorithm. Then, I tabulated the English words and assigned them one of several emergent semantic subcategories. Finally, I identified several representative lyrical samples for most of the subcategories and tabulated those separately. In the French-Canadian context, Shazam failed to distinguish between

Canadian provinces, allowing popular English songs to overshadow less accessible French works. Only two French-Canadian songs charted, which will motivate a case study for the Canadian genre sphere with lyrical data from the Québécois rapper FouKi. With two complete and regionally distinct datasets that inform the nature of these rappers' usages of English, I will place these borrowings within their sociocultural contexts and illustrate a unified rhetorical significance.

## Analysis: Categorizing and Contextualizing English Borrowing

I argue in the case of France that while outright English code-switching is rare, English borrowings into French constitute a set of incredible semantic variety. I attempt to portray this depth with my lexical subcategorizations and subsequent analysis of select translated lyrical contexts. In Canadian French rap, I demonstrate that English appears in a much more advanced, freeform manner, either in isolation, as a matrix language with embedded French, or as a subordinate embedding in dominant French. In both genre spheres, I claim that as creative deviations from prescribed French, these English weavings constitute a unique resistance discourse by which rappers reclaim power from the dominant culture and assert nuanced, translanguistic identities. To build this argument, I will first present the English borrowing practices in each country from a theoretical perspective and then inject the social significance.

## France: Qualitative Lexical Subcategorization and Contextual Analysis

Figure 1 provides my eleven novel subcategorizations for the English borrowings I observed on the mainland French rap charts as well as the words that informed each of them. To substantiate the nature of the practice for the reader and demonstrate its immense semantic variety, I will deconstruct several examples in their lyrical contexts. This first lyrical excerpt from "Méchant" by Niska contains borrowings from both the Violence and Bravado categories:

<i>Téma ma gueule, téma <u>mon gun</u>, téma ma caisse j'sais qu'ils la veulent</i>	Check my face, check <u>my gun</u> , check my car I know they want it
<i>Tout pour le fric, niquer le <u>buzz</u>, dix kilos d'<u>shit</u>, dix kilos d'<u>beuh</u> [11]</i>	All for the money, fuck <u>the buzz</u> , ten kilos of weed, ten kilos of herb [chart rank #18, Violence and Bravado]

Mixing multiple categories of borrowing within one couplet was a rare act within this lyrical corpus, but these acts demonstrate the comfortable command that rappers like Niska have over English as it pertains to their practice. Here, we see two English nouns with French articles, which is arguably the simplest form of borrowing. However, even this simple syntactic basis yields deep thematic contrast; each borrowing contributes to the unique rhetorical purpose of its surrounding line while respecting the French flow. The first line mixes integrity assertion and materialistic gloating, while the second line establishes Niska's value of dedication. Frameworks such as Paine's useful versus ornamental cannot capture

Lexical Subcategorization	Matched English Borrowings
Rap genre self-reference	lyric, flow, beat, (rap) game, rapper, remix, track, music, freestyle
English curse words	fuck, bitch, shit, [diminutive N-word]
Materialism	ice, jack, jet, street wear, Air Max, money, cash, Off-White
Bravado	hardcore, boss, buzz [noun], buzzer [verb], king, top, numbers, rookie, god, gossip
Drugs	joint, mule, smoke, dope, coke, dealer [verb]
Women	baby, wife, curly, mama, bitch, la miss, kiss
Violence	(high) kick, uppercut, shooter [noun, verb], kill, gun, street fight
Marginalized <i>banlieue</i> life	street, gang, hood, skatepark, school, alien, life, night, business, story, showcase, ghetto youth, team
Miscellaneous English slang	fire, bae, cool, daddy, delete [verb: un-add]
Reinterpreted English idioms: literal French translations of English idioms	on pull up, donner le go, trop clean pour toi, faire le move, être love de, tu la love, ma best life, on/elle dead ça, une speed life, avoir le time, avoir le blues, fuck la vie d'artiste
Complete English code-switching	what you gonna do, big up, what's his/your name, to the top, don't know yet, (let's) get it, finish him, yeah that's great, call me, Hood Star Beats

**Figure 1:** Notable English borrowings as located in 52 songs of Shazam's Hip-Hop/Rap Top 100 chart in France from the week of April 8<sup>th</sup>–15<sup>th</sup>, 2022, with my own subcategorization and notes.

this contrast, which necessitates subcategorizations like mine. This next example from “Chic choc” by Bolémv feat. Koba LaD includes an English slang verb and a noun borrowing referring to a woman:

<i>On s'verra p't-être plus tard, j'<u>delete</u> si tu réponds pas (Aye, aye, aye)</i>	We'll maybe see each other later, I'll <u>delete</u> if you don't respond (Aye, aye, aye)
<i>P'tite <u>curly</u> (<u>Curly</u>), bien gé-char, comme j'les aime trop, gars (Comme j'les aime trop, gars, putain) [12]</i>	Little <u>curly</u> ( <u>Curly</u> ), intoxicated, how I love them so much, man (How I love them so much, man, damn) [chart rank #5, English slang and Women]

While the prior example solely involved noun borrowings, Koba LaD's verse includes a present-tense borrowing of the English verb “delete.” The fact that the French clause it begins continues in the present tense demonstrates the value of retaining grammatical consistency even when mixing languages. Just as in the prior example, the articles or pronouns that borrowings assume as necessary are in French, which may reflect a wish to fluidly integrate English words. Together, these nuances point to English borrowing in mainland French rap being an intricate yet systematic practice. However, it shares the pitfalls that concern the rap genre as a whole; the second noun borrowing above is objectifying, reducing women to an archetype by hairstyle.

Now, I would like to progress into even more advanced borrowings on the mainland France charts. This first example from “Attentat” by PLK contains one of the many reinterpreted idioms I observed, which I define as literal French translations of English idioms. The resultant expressions do not exist in French, which led me to categorize these French expressions as English borrowings despite the French content that pervades them.

<i>Donne-moi ton numéro, donne-moi ton Snap' (yeah, yeah)</i>	Give me your number, give me your Snap (yeah, yeah)
<i>Tu m'donnes le go, t'inquiète, on s'capte (tu m'donnes le go) [13]</i>	<u>You give me the go-ahead</u> , don't worry, we get each other ( <u>you give me the go-ahead</u> ) [chart rank #70, Reinterpreted idioms]

Depending on perspective, reinterpreted idioms are either one step before (yielding more continuity than) or beyond (built upon) complete code-switching. They emerge out of another code, yet rappers mask the gravity of the borrowing by translating it back into the dominant code. This additional translation affords blending along phonetic and morphemic lines, among others; PLK blends by reusing the French verb *donner* from the prior line. There is a balancing act here, as the decision to break into a complete English

phrase constitutes automatic rhetorical significance. My last lyrical excerpt from “Dans ma paranoïa” by Jul showcases this reality:

<i>Dites-moi si j'ai changé, dites-moi si je suis plus le même</i>	Tell me if I changed, tell me if I'm no longer the same
<i>Dis pas que tu veux me teste, non mais attends là, <u>what's your name?</u></i> [14]	Don't say you wanna test me, no but wait, <u>what's your name?</u> [chart rank #51, Complete code-switching]

Hearing this excerpt, either as performed or faithfully read, immediately makes the abrupt transition from French to English apparent. In this way, the complete code-switch disregards subtlety and outright defies French preservationist values. I only observed it in 10 of the 100 songs, with only five examples total occurring mid-verse, rather than in producer tags or intros. The utter rarity of the practice in mainland French rap reflects a social stigma whose severity demarcates the genre sphere, since it is exactly the code-switch that defines Canadian French rap.

### Canada: A Case Study of the Fluid.Code-Switching of Lyrical Franglais

While the borrowings on the mainland French rap charts were occasional and usually limited to noun phrases, the Franglais in “Copilote” and “Bijou” by FouKi rapidly alternates within the spectrum of French, English upon dominant French, French upon dominant English, and complete English. Drawing from White's approach in accordance with the Matrix Language-Frame model, I counted occurrences of each category of Franglais in each song.

	“Copilote” by FouKi	“Bijou” by FouKi
<b>French</b>	26 (59%)	47 (77%)
<b>French-dominant</b>	14 (32%)	7 (11%)
<b>English-dominant</b>	1 (2%)	6 (10%)
<b>English</b>	3 (7%)	1 (2%)

**Figure 2:** Per-category linewise summations of Franglais as it appears in FouKi's rap lyrics.

The distribution of Franglais between the two songs is remarkably different. “Copilote” exhibits French-dominant code-mixing and English code-switching three times more often than “Bijou,” but displays one-fifth the frequency of English-dominant Franglais. Well beyond the margin of error, each category distribution reflects conscious stylistic intentions, through which FouKi modulates the relationships between the two languages. Further research could illuminate these intentions, perhaps through direct interviews with rappers like FouKi. Either way, even this limited data shows that Franglais is not a monolithic practice, that it can assume many forms

under one speaker depending on the context. To demonstrate how the intermediary forms of Franglais take shape, I have selected three lyrical examples for each of the French-dominant and English-dominant code-mixing categories. I will begin by analyzing French-dominant Franglais.

<u>Check-moi flex</u> si on s'croise dans rue [15]	<u>Check my flex</u> if we meet in the street [chart rank #41, French-dominant]
Tu fais partie des <u>cool kids</u> [15]	You're part of the <u>cool kids</u> [chart rank #41, French-dominant]
Tu peux pas <u>mess around</u> avec la troupe [16]	You can't <u>mess around</u> with the troupe [chart rank #57, French-dominant]

**Figure 3:** Excerpts and translations of French-dominant Franglais in FouKi's charting songs.

Interestingly, the expression “Check-moi flex” [15] from the first line constitutes a reinterpreted idiom, as seen in my earlier analysis. FouKi's reinterpretation plays upon standard French pronoun inversion, usually seen in imperative and interrogative forms, extending it to reflect the possessive form of the original English. The creativity continues in the second and third lines, in which FouKi inserts compound English slang borrowings within French sentences. One compound slang borrowing, “cool kids” [15], constitutes a noun phrase while the other, “mess around” [16], is a verb phrase. On the mainland French rap charts, I hardly observed compound borrowings, with not a single compound verb phrase outside of complete code-switching. This screams that even the simplest acts of lexical borrowing may take on more advanced forms in the context of French-speaking Canada, where English and French language cultures overlap more strongly.

At other moments in his rap performance, FouKi constructs Franglais from an English basis, inserting French noun phrases or dependent clauses that respect high-level English syntax. These three examples from the same songs demonstrate variety even within this category.

She used to be <u>ma copilote</u> [15]	She used to be <u>my copilote</u> [chart rank #41, English-dominant]
Watch out <u>quand le beat il joue</u> [16]	Watch out <u>when the beat plays</u> [chart rank #57, English-dominant]
Good times <u>que nous accumulons</u> [16]	Good times <u>that we accumulate</u> [chart rank #57, English-dominant]

**Figure 4:** Excerpts and translations of English-dominant Franglais in FouKi's charting songs.



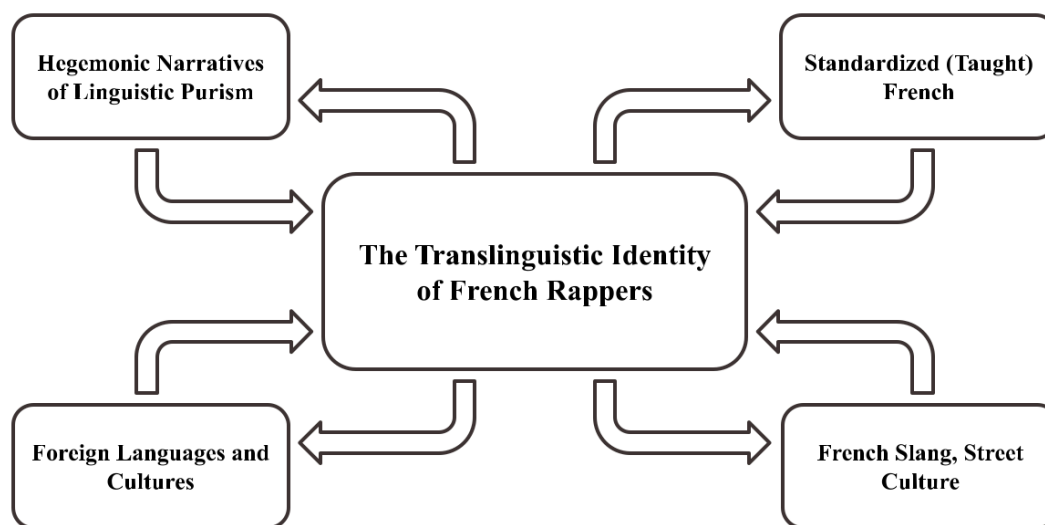
Recall that “Bijou” produces a fivefold increase in English-dominant Franglais over “Copilote.” It is also the song with more adventurous French embedding, containing multiple examples of French dependent clauses in English sentences. The fact that French can serve as a subordinate language to English in these Canadian rap lyrics brilliantly illustrates the crux of the resistance discourse: challenging French linguistic purism by modulating the dynamics of borrowing.

### Social Significance: English Borrowing as a Creative Resistance Practice

Clearly, a highly creative and dynamic practice has emerged around English borrowings in French rap, both in mainland France and in Canada. However, it is exactly the kind of practice that gets rappers denied \$18,000 grants and prevents their music from being played on French radio stations. These examples reflect the linguistically prescriptive pressures that the dominant culture in these genre spheres exerts upon French rappers. Despite these pressures, rappers

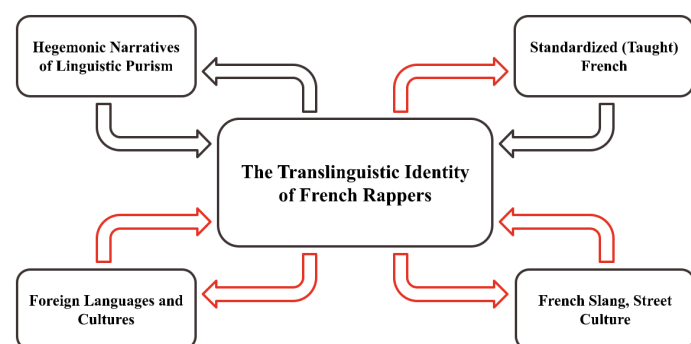
modulate the default, standardized French, taking influences from street slang and foreign languages via deliberate, culturally aware choices. I argue that English language borrowings constitute a significant part of this emergent resistance discourse by which rappers challenge narratives of linguistic purism and, in doing so, construct translinguistic identities.

Post-colonial studies scholar Lara Dotson-Renta captures the exigence fueling the genre: “French hip-hop frequently contests and examines the contours and parameters of a national French identity, proposing that not everyone within France shares the same relationship to the historical legacies and events upon which French national ideals were built” [17, p. 354]. Since standard language is inexorably bound to historical legacy, French rappers convey their distance from that legacy when they defy standardizations. At the same time, every act of borrowing or slang construction communicates a relationship with an alternate language culture, reclaiming power by platforming marginalized communities such as those within *les banlieues*.



**Figure 5:** Mapping the sociolinguistic forces at play in the identity formation of French rappers.

### Conclusion: Why It Matters to Empower This Non-standard Language Use



**Figure 6:** Sociolinguistic negotiation processes threatened by institutional linguistic purism.

As linguistic prescriptivism prevails, many aspects of how French rappers construct and affirm their identities hang in the balance. Within their commercialized genre, these rappers must negotiate the wavering dynamics of the music industry, in which narratives from the dominant culture define mass marketability. Additionally, linguistic purism serves to cut off the practice of language change as it emerges from French rap music, marginalizing the generations who adopt its language practices. Prohibiting these foreign and domestic influences into French which more accurately reflect the cultures of interlocutors can only further dysphoria within French identity. This act exacerbates preexisting oppression, and it forcefully limits the dimensions of creative expression within a language so admired for that very artfulness. Within their societies that deplatform emerging language practices and paint them as deviant, French rappers emerge as defenders of an inclusive future for their communities. I urge readers to recognize their dynamic music genre

for what it truly is: art for a complex, diverse French language diaspora, not a stain upon some contrived linguistic heritage.

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# Unraveling the Legacy: The Impact of Soviet-Era Political Violence on the Russo-Ukrainian Conflict

**KSHITIJ SINGH**

*TRINITY COLLEGE DUBLIN*

This paper attempts to answer the research question: To what extent has the ongoing Russo-Ukrainian conflict since 2014 been catalyzed by the legacy of political violence in the USSR under Bolshevik leaders like Lenin and Stalin? The research explores historical events, such as the Ukrainian-Soviet War, the Holodomor famine, and systemic Russification during the Stalin era, and how these have nurtured anti-Russian sentiment and far-right elements within Ukraine. Through historical analysis, this study identifies how the rejection of Ukrainian identity and the forced assimilation into a Soviet identity have contributed to the present-day conflict. Ultimately, the analysis argues that these historical grievances have fueled Ukraine's drive towards EU and NATO membership, exacerbating tensions with Russia and contributing to the escalation into all-out war. However, the paper also acknowledges the role of contemporary geopolitical factors, such as NATO expansion, in driving the conflict. The findings suggest that while Soviet-era political violence has played a significant role, modern dynamics, and NATO activities are also to blame. The implications of this research underline the importance of forming inclusive alliances focused on limiting territorial expansion and competition and understanding the risks inherent to state relations shaped by significant power imbalances.

**Keywords:** Soviet Political Violence, Russo-Ukrainian Conflict, Russification, Ukrainian Nationalism, Far-right politics, NATO, Genocide

## Introduction: Historical Background

The historical roots of the modern-day Ukrainian and Russian state date back to the 9th-century medieval federation of Slavic tribes known as Kievan Rus [1]. Following the Mongol invasions in the 13th century, however, this federation fragmented, and the territories of modern-day Russia and Ukraine began to develop different trajectories. Most of what is today western Ukraine came under the Polish-Lithuanian Commonwealth in the 14th century, which shaped its distinct culture and identity in contrast to territories in the east. Meanwhile, the Grand Duchy of Moscow expanded his territory, and by the end of the 18th century, much of eastern and central Ukraine was incorporated into the Russian Empire after the decline of the Polish-Lithuanian Commonwealth [1]. The 19th century brought a wave of Ukrainian nationalism spearheaded by intellectuals who emphasized distinct Ukrainian identity and heritage, even as the region remained under foreign rule. With the Russian Revolution in 1917, movements for autonomy emerged in modern-day Ukraine, resulting in the creation of the Ukrainian Central Council, which advocated for autonomous governance of Ukraine without separation from the Russian Republic [1]. This council announced the creation of the Ukrainian People's Republic (UPR), which was recognized by the Russian Provisional Government. However, with the Bolshevik seizure of power, the UPR rejected Soviet ideals and vowed to combat coup attempts within Ukraine. Soon after, Bolshevik groups in Kharkov declared war against the UPR, announcing the creation of the

Ukrainian People's Republic of Soviets, thereby commencing the Ukrainian-Soviet War [2]. The war eventually led to Bolshevik victory and integration of Ukraine into the USSR by 1922. Under Lenin, while the Ukrainian language and culture was allowed to exist, economic turmoil swept through. Then, under Stalin, conditions worsened. Political repression, forced labor, man-made famine, and deportation followed [2]. After the collapse of the USSR in 1991, Ukraine decided to become a non-aligned state, seeking closer ties with the European Union (EU) and undergoing periods of tense relations as well as cooperation with Russia.

In 2014, the Revolution of Dignity in Ukraine resulted in the ousting of pro-Russian president Viktor Yanukovich from power, catalyzing a political vacuum which led to significant political instability and eventually culminated in the Donbas conflict [3]. During this, separatist forces, supported by the Russian government, marched into the eastern regions of Ukraine and captured key infrastructure. Soon after, Russia annexed Crimea in a referendum in 2014 and the Donbas War transformed into a static war [4]. This was sustained for the next eight years until February of 2022, when the small-scale conflict descended into an all-out war between Russia and Ukraine. This was prompted by the entry of Russian forces into Ukrainian territory on the pretext of "denazifying" [5] Ukraine and conducting a special military operation to support the Donbas separatists. International condemnation followed swiftly, demanding complete Russian withdrawal from the region. Despite this, Russian President Vladimir Putin continued to espouse irredentist views,

pointing to the historical and “spiritual” [5] proximity that binds Ukraine to Russia, alongside claims of a *volonté générale* to realign with Russia given recent ‘referendums’ in the nation’s eastern provinces. In this way, underpinning the operation are arguments grounded in democratic legitimacy and historical fraternity, both of which make the war rage on with no clear end in sight.

In this light, this paper posits that, to some extent, the Russo-Ukrainian war has been catalyzed by the Soviet legacy of political violence. This will be illustrated through two main components. First, that anti-Russian sentiment and far-right presence in Ukraine which initially justified the need for Russian invasion was catalyzed by a reaction to the USSR’s undermining of the former Ukrainian Soviet Socialist Republic. Second, the rejection of Ukrainian identity as a means of inculcating a Eurasian Soviet identity has produced a modern-day Russophobic nationalist movement. This has subsequently propelled EU and NATO membership talks with Ukraine and thus prompted the military invasion. These two points will proceed in the order outlined above, by firstly examining the atrocities committed by the Soviet regime and their impact on fostering anti-Russian and far-right sentiment within Ukraine. Then, the Soviet Union’s systemic suppression of Ukrainian identity will be discussed, along with how it has shaped modern-day Ukrainian policy towards the Russian government. Finally, the role of contemporary geopolitical tensions between NATO and Russia will be expounded upon, offering a more nuanced understanding of the several factors that have fueled the war beyond historical sentiments. With this, it will be argued that while the legacy of Soviet political violence has played a fundamental role in shaping the Russo-Ukrainian conflict, its influence has been limited to some extent, as current geopolitical dynamics have acutely triggered the conflict.

### **From Famine to Genocide – Producing Discontent in Contemporary Ukraine**

Firstly, the Russo-Ukrainian conflict has been catalyzed to a significant extent by the legacy of Soviet political violence, as the atrocities under the USSR cultivated Russophobic sentiment within Ukraine and consequently fomented hostility between the states. This is exemplified particularly by two famines: the 1921-1923 Ukrainian famine and the Holodomor famine. In 1921, along with other regions of the USSR, the region of Ukraine suffered from severe drought, leading to widespread starvation and thousands of deaths. During this, the Soviet government, led by Lenin, decided to provide food aid to other regions by transferring grain from Ukraine. The Ukrainian regional government, “starving themselves, were called upon to supply grain to relieve the Volga region” [6]. Similar to this, again, in 1932, the famine known today as the Holodomor famine was not only ignored in Ukraine by the Soviet government but also manufactured by it. Stalin’s government had embarked on rapid collectivization, and this caused a food shortage due to declining agricultural output. Between 1932 and 1933, the Soviet regime attempted to provide relief, but doing so while discriminating against Ukrainian ethnic regions as “Ukrainian populated areas were given a lower number of tractors” [7] and simultaneously implementing policies that historian Timothy Snyder labeled as “administrative measures...to kill” [7] rather than aid the population. As such, the Bolshevik administration actively discriminated against the Ukrainian regions in two significant

instances of political violence, carrying out a Russocentric agenda instead. The long-term legacy and consequence of this has been the development of radical anti-Russian sentiment and fragmentation of Russia-Ukraine relations [8]. This hatred of Russia has been “set in stone” [9] to such an extent that the Svoboda and Right Sector, which are far-right ultranationalist groups with Neo-Nazi elements, played a significant role in the 2014 Revolution of Dignity because of a general dislike of “any civilized relationship with “Russia” [9]. During protests, they tore down a Lenin statue, and yelled anti-Russian slogans alongside the famous “Hang the Commie” [9] chant. This demonstrated the hatred possessed by specific Ukrainian groups against the Soviet regime, but more significantly, displayed the “trauma” [10] which “haunts” [11] Russia-Ukraine relations. This Russophobic and ultranationalist presence with fascist ideology has provoked Russia to claim that Ukraine is run by “Neo-Nazis” [12] who pose a threat to Russian existence and therefore must be defeated [13]. With this, the legacy of political violence in the USSR can be seen, as the discrimination of the Ukrainian regions sowed long-lasting resentment towards Russian influence, so much so that extremist right-wing groups gained traction in Ukraine. In turn, Russia responded by framing its military operation as a necessary measure to protect Russian-speaking populations from these ‘fascist’ elements. While it is difficult to comment on the legitimacy of all Russian claims, it is clear that escalating tensions rooted in historical grievances played a prominent role in triggering the conflict. Therefore, it can be argued that political violence under Bolshevik leaders of the USSR has left lasting trauma, fostering the emergence of far-right elements in Ukraine, subsequently contributing to heightened hostility between the Russian and Ukrainian states. As such, the legacy of Soviet political violence has catalyzed the Russo-Ukrainian conflict to a significant extent.

### **West or East? – The Ukrainian Dilemma**

Secondly, the rejection of Ukrainian identity as a means of inculcating a Eurasian Soviet identity under the USSR has had a significant role in propelling EU and NATO talks with Ukraine, thereby instigating the Russo-Ukrainian conflict. The evidence of this lies in the systemic elimination of the Ukrainian language and identity in the 1930s and 1940s under Stalin, who embarked on a policy of Russification. This was exemplified by his telegram titled “Stop Ukrainization” [14] which coincided with the expulsion of 84,653 members of the Ukrainian branch of the Communist Party and their replacement with Russians from other regions [14]. Alongside this, Russian became the primary language for administrative tasks, and the usage of the Ukrainian language was condemned in schools and offices [15]. Moreover, later on, the regime deported around 571,000 Ukrainians between 1940 and 1953 on the basis that they were “enemies of the people” [16]. The consequence of such repression and violence was the fomentation of “strong anti-Soviet sentiment that persisted through generations” [17]. The resentment directed towards the “Muscovite, Russian rule” [17] was exacerbated by the feeling that said violence was carried out less for “their behavior than for their identity as [ ... ] Ukrainians” [17]. As scholars Rozenas, Schutte and Zhukov illustrate, this bitterness has sustained to this day, given that “anti-Russian political preferences” [17] are most intense in regions where Soviet repression and violence was the most extreme. Consequently, it would be reasonable to state that the legacy of the



USSR has given rise to hostile Ukrainian attitudes towards Russia. The culmination of this was most notable in 2014, when President Yanukovich was ousted predominantly because of his decision to choose closer ties with Russia and not sign an Association Agreement with the EU [18]. With his downfall, the new Ukrainian administration moved even closer to the EU, marked by the signing of the EU-Ukraine Association Agreement and multiple amendments to the constitution to make Ukraine suitable for EU membership. Furthermore, Ukraine also renounced its non-aligned status and made joining NATO a main foreign policy objective despite Russian condemnation [19]. The Russian administration demanded NATO ban Ukraine from joining and “end any further NATO moves eastward” [20]. With the rejection of such demands, tensions escalated to the point of full-scale war in 2022, a war which became feasible partly due to Ukraine's desire, fueled by its historical animosity towards Russia from the Soviet era, to move incrementally closer to the EU. That said, while there were several causes of the conflict, the historical legacy of political violence in the USSR transformed the political landscape in Ukraine, nurtured anti-Russian elements and encouraged Ukraine to look westward, making it a significant factor in catalyzing the Russo-Ukrainian conflict.

### Putin and NATO's War

On the contrary, it can be said that the Russo-Ukrainian conflict since 2014 has been catalyzed not so much by the legacy of political violence in the USSR, but by a clash between Russian and NATO expansion. Proponents of this view contend this in light of the fact that the majority of Russian claims regarding the presence of Neo-Nazis in Ukraine have been labeled false or gravely exaggerated [21]. Ukraine does have “right-wing extremists and violent xenophobic groups” [22] but that is the case for almost every country in the world, including states bordering Russia. Adding to this, historian Timothy Snyder has presented the perspective that the Russian regime makes such statements in order to “justify unprovoked war” [23] through political aesthetics that could be accepted in the western world. This would demonstrate that the legacy of political violence in the USSR has had little real influence in catalyzing the Russo-Ukrainian conflict because the reasons pushed forth by Russia have not prompted the invasion. Rather, Russian jingoism, driven by Putin's government, could predominantly be at play. Moreover, the expansion of NATO appears to be significant in provoking Russia, a provocation having little to do with the legacy of political violence in the USSR. After the Soviet Union collapsed, Russia, under both Vladimir Putin's administration as well as his predecessor Boris Yeltsin's, claimed that informal assurances had been made by NATO leaders to not expand eastward, which they had consistently violated by adding more Eastern European countries to the fold [24]. While the legitimacy of these informal assurances has been debated, scholar Marc Trachtenberg has suggested that the Russian argument was “by no means baseless” [25]. At the same time, in response to said NATO enlargement, Russia has expanded its own territory, participating in the 2008 Russo-Georgian War and annexing Crimea in 2014 [26]. Since then, the geopolitical importance of Ukraine for both NATO and Russia, as well as its historical and cultural significance for Russia, has led to Ukraine becoming a battlefield for both these parties. This perspective was supported by close advisor of President Putin Sergey

Karaganov, who stated that NATO exclusion of Russia “automatically put Russia and the West on a collision course, eventually sacrificing Ukraine” [27]. Additionally, recent developments have shown Ukrainian President Volodymyr Zelensky is willing to forgo some of Ukrainian territory to Russia in exchange for NATO membership [28]. The mere fact that Ukraine is considering such a concession highlights the extent to which NATO membership and long-term security have been central to Ukraine's playbook. Meanwhile, Russia has made clear its dissatisfaction with any future plans for Ukraine's NATO integration [29]. This shows that the confrontation between Ukraine and Russia since 2014 has not only stemmed from anti-Russian sentiments in Ukraine or the overall legacy of political violence in the USSR, but also from NATO expansion, which has bolstered its sphere of influence in Eastern Europe with little regard for Russian concerns. As such, the Russo-Ukrainian war has been catalyzed by the legacy of Soviet political violence, but only to some extent.

### Conclusion & Implications

Overall, this paper argues that to some extent, the Russo-Ukrainian war has been catalyzed by the legacy of Soviet political violence under leaders like Lenin and Stalin. This is primarily because of two factors: first, the historical repression and atrocities committed under the Soviet regime entrenched deep-seated anti-Russian sentiment in Ukraine and bolstered Ukrainian nationalism. This was demonstrated through the 1921-23 Ukraine famine as well as the Holodomor famine, during which Ukrainian regions were not only discarded but deliberately targeted. The historical trauma and legacy of these tragedies has shaped Ukraine's hostility towards Russia and empowered far-right nationalist elements within the country; second, the inculcation of Eurasian identity during the Soviet era distanced Ukraine from Russia and instigated its push towards the Western bloc, further compounding geopolitical tensions. Russification policies during Stalin's rule aimed at systematically eliminating Ukrainian identity and language, left long-lasting bitterness which consequently fueled Ukraine's desire to actively reject Russian influence. However, these historical factors did not solely determine the trajectory towards conflict. The clash of NATO expansion with Russia's strategic interests also played a prominent role. NATO's expansion in Eastern Europe was perceived as a betrayal of informal assurances and most importantly a threat to Russia's sovereignty. In such circumstances, the justifications based on Neo-Nazism or far-right extremism, which have been invoked by the Russian government to rationalize their actions in Ukraine, become less conclusive. As such, while the Russo-Ukrainian conflict is a culmination of historical grievances and political violence during Soviet times, it is also significantly shaped by contemporary geopolitical struggles involving Russian and NATO forces.

This investigation yields two primary implications. Firstly, it underscores the vulnerability of newly formed states not only to political instability, but also unintended manifestations of unresolved historical grievances. In the case of post-Soviet Ukraine, a history of violence, along with the lack of mutual and egalitarian cooperation, guided strong anti-Russian sentiment and created internal discord. At the same time, relentless competition between NATO and Russia engulfed Ukraine and fractured relations in the region for decades to come. This implies the need of inclusive coalition-building and cooperation between Russia and the Western

bloc to address issues of territorial expansion in order to ultimately resolve the conflict. A definitive mutual agreement to eliminate interference in the domestic affairs of other states and cease territorial expansion on both sides could be effective in de-escalating tensions and ameliorating historical grievances. Secondly, this paper demonstrates the risks of asymmetric power dynamics in diplomacy, where one state asserts considerable influence over another both economically and culturally. This indicates the need for a more cautious approach focused on equitable negotiation and respect of absolute sovereignty, especially when dealing with newly created states that could perceive external influence as a threat to their autonomy.

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# A Different Lens for the Earthworks Experience

**LAURA MACKENZIE**  
**CORNELL UNIVERSITY**

The Earthworks movement of the 1960s and 70s challenged the conceptual art world by transforming nature into a gallery space. Land artists manipulate existing landscapes, using nature as a medium, to create large-scale works bound to their sites. Both critics and artists have stressed the experiential nature of such work, and as a result, the dominant process for viewing these installations is to visit them in person. Through an analysis of land artist Nancy Holt's photographic piece titled *Sunlight in Sun Tunnels*, this paper reconsiders the most effective method in which viewers interact with Earthworks. Analysis of Holt's work reveals the ways in which the medium of photography alters the Earthworks experience and proposes its use as a more powerful alternative to traditional means of viewing.

Earthworks, also referred to as Land Art, is a conceptual art movement of site-specific sculptures that gained momentum during the 1960s and 70s. Early Earthworks were defined by their manipulation of remote wilderness, often through the excavation or reshaping of an existing landscape [1]. The art form has expanded to include any work bound to its site, which has allowed for the incorporation of man-made materials such as concrete and steel into these projects [2]. The movement's popularity during the 60s and 70s can be attributed in part to the public's increased interest in protectionist environmentalism, a movement that hoped to prevent the disruption of natural ecological systems through the regulation of chemical and land use, as opposed to traditionalist genteel conservation [1]. During this time period, many Land Artists used terrain and ecology as their canvases to add to the discourse about human's relationship to nature. Others found themselves "disenchanted with the commercialism of the art world" and turned to Earthworks as a rejection of capitalist values and culture [2].

In her artwork *Sunlight in Sun Tunnels*, Land Artist Nancy Holt utilizes the medium of photography to document one of her most well-known installations. The art piece is titled *Sun Tunnels* and consists of four large-scale concrete cylinders strategically placed in an 'X' on the desolate surface of Utah's Great Basin Desert. Two of the cylinders frame the sun at sunrise and sunset during the summer solstice, and the other two during the winter solstice. Holt brings more of the cosmos into her sculpture by drilling holes that mimic constellations through each of the concrete shells. For her piece *Sunlight in Sun Tunnels*, Holt photographs one of these concrete cylinders every half hour over a span of fifteen hours then arranges the images chronologically into a gridded pattern [3, Fig. 1]. The resulting photograph allows the viewer to trace the sun's interaction with the site and sculpture throughout the day. Holt's installation changes the way people experience the site, and the composite photograph shapes the way viewers experience the sculpture. Photographic projects such as *Sunlight in Sun Tunnels* contradict the assumption that visiting the site is a requirement for experiencing an Earthwork.

Land Art compels the viewer to reconsider their relationship to the world around them by introducing sculptural

features into the landscape. Land Artist James Turrell explains his approach to the medium as being "concerned with what my spaces direct their seeing to, and hence what they direct our seeing to" [4]. The placement of Holt's sculpture invites the viewer to peer through the large concrete tubes, quite literally framing the sun as it rises and falls above the horizon line, like a magnifying glass bringing a piece of the landscape into focus. In *Sunlight in Sun Tunnels*, the use of negative and positive space directs the viewer's eye toward the horizon just as successfully. The dark shadows that consume much of the photograph act as negative space, bringing the viewer's attention to small circles of positive space at the center of each individual frame. The contrast between the dark shadows and faded landscape enhances the viewer's focus.

The use of photography in documenting these works challenges conventions of how and where Land Art is meant to be experienced. Earthworks are often heralded as "perceptual and experiential" artworks that rely on the "involvement of spectators." Many scholars and art critics feel that visiting an Earthwork in person creates a nuanced relationship between human, sculpture, and site that is not available in art galleries [2]. Land Art is a form of conceptual art which appeals to the elite art sphere, but the concept of reexperiencing the outside world also has a more universal appeal. Earthworks evoke innate human emotions by manipulating the spaces they inhabit. For instance, the distinction between bound spaces, which "enclose firmly by clearly delineating spatial boundaries" and unbound spaces, which are "more open and expansive," create different experiences for users. The former establishes feelings of comfort and security while the latter evokes a sense of freedom [5].

Land Artists' strong use of scale is one possible explanation for these sentiments. According to Renshaw, large-scale Earthworks "render the human spectator insignificantly small" and allow viewers to "reexperience nature's vast scale" [6]. In *Sunlight in Sun Tunnels*, Holt is able to continue this exploration of scale through a photographic medium instead of a sculptural one. At the center of each frame, the far end of the sun tunnel creates a small, circular window into the vast Great Basin Desert. The dark shadows that fall inside the tube take up the majority of the photograph, while the





*Hot Sunlight in Sun Tunnels, 1976*

actual landscape is confined to a snippet of barren desert scrub against a faint blue sky. This makes the desert feel distant, even though it surrounds the entire sculpture. Within the shadows lie scattered light gaps that resemble constellations, and in the foreground of the last few images shines a funnel of golden light which indicates the sun's presence. The scale of these astronomical elements in comparison to the desert landscape makes the viewer feel closer to the cosmos than to Earth.

Holt's photographic representation of her Earthwork may be a reproduction of her large-scale sculpture, but it does not lose its ability to communicate the temporal or atmospheric qualities of the physical artwork. Holt's composite photograph is more than mere documentation of her installation. In a review published in *Art Monthly*, Cherry Smyth describes how Holt's photography brings to life the more dramatic aspects of her sculptures, allowing her to recreate the site's atmosphere in two-dimensional space [7]. The repeating circular shapes and curved, eclipsing shadow that can be

tracked throughout the piece are reminiscent of space photography, which mimics the celestial experience of the physical installation.

In addition to capturing the atmospheric qualities of a piece, photography is a powerful tool for artists who seek to capture the transformative nature of Land Art. Earthworks are often designed to be altered by site-specific conditions and processes. Sculptures located in the desert, for instance, are subject to weathering and deterioration that does not exist in a gallery setting. A prime example of this phenomenon is a pair of photographs taken ten years apart of Robert Smithson's *Partially Buried Shed*. The photographs show the evolution of a small shed as it collapses under the weight of piled dirt. The installation is located on Kent State's campus, so human forces played a large role in the work's transformation as well, especially after a student demonstration and tragic shooting that occurred in 1970. The painted words "MAY 4 KENT 70" captured in the second photograph serve as a reminder of the site's painful history [8]. For many land artists, this



transformation over time is as much a part of the piece as the initial sculpture. Horning explains how this type of photographic record is not only a “history of the work as [an] object” but that it also has the potential to become a “photographic subject that may outlast the work” [8]. Therefore, the purpose of photographing an Earthwork is twofold; It documents the changing installation, but also produces a new art piece that captures more than what is communicated by the installation at its initial completion.

Holt’s composite photograph *Sunlight in Sun Tunnels* achieves both functions. The sculptural tunnels are temporal due to the way they are visually altered by their interactions with the sun. The photograph’s symmetrical framing of the tunnel remains the same in each shot in order to bring the viewer’s attention to this dramatic shifting of light and shadows. In the first few frames, an orange hue warms the smooth concrete as the sun rises. The concrete transitions to a cooler gray as the sun makes its way across the sky, until the inside of the tube becomes fully submerged in shadow and appears black to the viewer. In the final row of images, the sunset creates a golden glow on the inside of the tunnel. The viewer is exposed to the Earthwork’s full cycle of transformation, which would not be possible if they were to visit the site in person for an hour or so. Holt’s composite photograph becomes an adjacent yet separate work from the sculpture by allowing the viewer to experience the temporal and cyclical relationship between the earth and the sun all at once.

Despite the movement’s emphasis on the in-person experience, photographic representations of Earthworks appear in museums for a multitude of reasons. This is contradictory to the aims of many modern Land Artists, who believe it is important to move away from a more “private and intellectual” scene and involve an “increasingly democratic and non-elitist” audience [2]. The movement is a subversion of the elitist art scene where middle- and working-class citizens are “denied an understanding and appreciation of art that would allow them to be a part of museum culture” due to governing bodies being made up of strict groups of elite citizens and their use of “arcane language familiar to those schooled in art” [9]. Land Art is accessible because it is conceptual work with a more universal appeal, as mentioned earlier, but it is also increasingly available to a broad variety of social classes because it exists outside the physical boundaries of a gallery space.

Even with these boundaries removed, however, many Earthworks are not very accessible to the general public because they occupy such remote sites. Holt’s *Sun Tunnels*, for instance, is located 40 miles from the nearest active town [6]. Photography provides a controversial solution to this issue because although it improves accessibility, it returns to the idea that art is a commodity to be bought and sold rather than something to be experienced. Some Land Artists embrace the movement as a rejection of this very idea. Boettger explains how their subversion is flawed because the creation of Earthworks depends upon “funding by art dealers, businesspeople, and art collectors, and documentation of these works was exhibited for sale in commercial galleries” [1]. Earthworks would be unable to exist without support from the elite art world and photography’s distribution properties, creating a complex relationship between the movement’s conflicting aims.

Although photography is not the intended lens for many Earthworks to be viewed through, it is the most functional one. Photography makes Land Art available to the masses while still

upholding the physical work’s commentary on humans’ relationship to the natural world. Furthermore, these themes are not only translated but also enhanced when Earthworks are treated as photographic subjects, as proven by Nancy Holt’s composite photograph *Sunlight in Sun Tunnels*.

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# “I Am Not Minor”: Unviable Redemption and Patriarchal Structure in *Disgrace*

AMY RUCKMAN

LOUISIANA STATE UNIVERSITY

J.M. Coetzee’s 1999 novel *Disgrace* has received extensive academic study, with many analyses assessing the merit of the main character’s “redemption,” the theme of animal rights, and the broader significance of the novel as an allegory for post-apartheid South Africa. While there is also literature that examines the novel’s displays of misogyny, there is a noticeable lack of research on the patriarchal structure that underlies the oft-discussed “redemption” present in *Disgrace*. As such, this paper argues that the theory of redemption *Disgrace* offers for the future of South Africa is undermined by its reliance on patriarchal structures. The novel indeed constructs a redemptive theory grounded in a moral shift, repentant spirituality, and personal sacrifice, allowing the story to end on a seemingly hopeful note. However, a closer analysis of David’s experiences and interactions with women, especially with Lucy, reveals how these redemptive arcs are facilitated primarily through patriarchal structures, relying on the erasure, silencing, and sacrifice of women; David refuses to respect Lucy’s decisions, lashes out at Dr. Farodia Rassool, and foregrounds Mr. Isaacs’ authority as he dismisses the feelings of Mrs. Isaacs and Desiree. The redemptive theory *Disgrace* offers is therefore unviable because it unethically constructs itself at the expense of women’s autonomy and suffering, in which any “redemption” demonstrated is fundamentally exploitative. Ultimately, *Disgrace* reaffirms the futility of imagining a more just post-apartheid world when this world continues to exploit women.

**Keywords:** *Disgrace*, South African literature, patriarchy, misogynoir, postcolonial literature, redemption

## Introduction

J.M. Coetzee’s *Disgrace* takes on a heavy task for a novel just over 200 pages: how can white South Africans make amends for the violence they perpetrated during apartheid? Some critics claim that *Disgrace* depicts white reformation as a “journey to personal salvation” [3], where the novel offers hope for social transformation through personal betterment. This paper, however, posits an alternate perspective. While *Disgrace* indeed constructs a redemptive theory grounded in a moral shift, repentant spirituality, and personal sacrifice, a closer analysis of David’s experiences and interactions with women, especially Lucy, reveals how these redemptive arcs are facilitated primarily through patriarchal structures, relying on the erasure, silencing, and sacrifice of women. The redemptive theory *Disgrace* offers is therefore unviable because it unethically constructs itself at the expense of women’s autonomy and suffering, in which any “redemption” demonstrated is thus fundamentally exploitative.

## Moral Shift and the Disregard of Women’s Knowledge

Over the course of *Disgrace*, David becomes attuned to a broader set of moral principles that place greater value on the nonhuman world. Feminist scholar Marianne DeKoven argues that his gradual attunement to these new ethics constitutes his “salvation narrative,” in which David can only embrace this capacious morality through dogs and *women*, especially Bev Shaw [3]. Thus, many critical texts

focus on the role of Bev Shaw in David’s redemptive journey, with another scholar explicitly calling her “[David’s] redeemer” [7]. However, *Lucy* is actually the first character to introduce to David this respect for the nonhuman, telling him that “[t]his is the only life there is. Which we share with animals.... [We must try t]o share some of our human privilege with the beasts.” In return, David refuses to recognize the significance of Lucy’s words, instead dismissing her with the rationale that humans “are of a different order of creation” than animals [2]. His disregard for Lucy’s perspective, especially juxtaposed with his gradual respect for Bev Shaw’s ethical approach to the world, suggests a misogynistic distinction perhaps on the basis of age but more likely on familial hierarchy. Though David may not see Bev Shaw as an “equal,” his choice to have an affair with her—compounded by the narrator’s dry commentary for David to “stop calling her poor Bev Shaw. If she is poor, then he is bankrupt”—at least suggests that David learns to recognize her individual personhood [2]. In contrast, the paternal hierarchy invoked regarding Lucy’s status as his daughter denies her this basic acknowledgment. Well into the novel, Lucy continually criticizes David for his refusal to hear her: “You have not been listening to me” [2]. David’s inability to recognize Lucy’s own capacious morality and thus her role in his ethical shift reflects historical patriarchal dominance of the father over the daughter, where the daughter’s wise words are perhaps unconsciously processed but remain effectively unheard.

The pattern of patriarchal dominance over Lucy repeats throughout the novel, contrary to the notion of redemption leading to self-betterment. Even as David appears to internalize the more inclusive morality that recognizes the intrinsic value of the nonhuman—suggested by his choice to cremate the euthanized dogs' corpses himself—he does not deign to offer such intrinsic respect to his own daughter [2]. “This is the only life there is,” and it is one David shares not just with animals but also with women. Yet, time and time again, he fails to recognize Lucy's autonomy. As Lucy herself later says: “You [David] behave as if everything I do is part of the story of your life. You are the main character, I am a minor character.... I am not minor. I have a life of my own... and in my life I am the one who makes the decisions” [2]. In this moment, she explicitly puts into words David's inability to view her as an autonomous being. His failure here marks a stark contrast to the same David who could not bear to eat two sheep after tending to them for a few days [2]. David can honor the individuality of two nonhuman animals, but not that of a woman in his immediate family. Of course, it should be noted that David's domineering behavior increases after Lucy's rape, arguably reflecting a genuine concern for his daughter's well-being. However, his assumptions regarding her experience of sexual violence undercut his concern: “But you weren't there [when Lucy was raped], David. She [Lucy] told me [Bev Shaw]. You weren't” [2]. In chafing against Lucy's decision to not share with him or the police the details of her rape and assuming he understands her experience *without* her telling him what happened, David further victimizes his daughter. His repeated attempts to control Lucy and her life thus beg the question: does “David Lurie's salvation narrative” truly place “the possibility of hope” in women [3]? Or does David exploit the wisdom of women for his own self-betterment without acknowledgment of their position as facilitators of his transformation—particularly that of his own daughter—and belittle them in the process? If the situation is the second, the unviability of *Disgrace's* redemptive theory becomes clearer because of its exploitative appropriation of women's knowledge and experiences.

### Repentant Spirituality and the Manifestation of Misogynoir

While the presence of women as facilitators of David's ethical journey can be read favorably or unfavorably, the spiritual element of *Disgrace's* theory of redemption is more clearly made possible only through patriarchal structures. Here, “spiritual” refers to the sincere apology, reflecting David's shift toward true remorse for his predatory actions against Melanie. Early in the novel, David displays an utter lack of repentance for his behavior. An oft-cited example is his refusal to apologize and genuinely acknowledge his wrongdoings at the university hearing. Importantly, Dr. Farodia Rassool is the only character to criticize David for his “fundamentally evasive” responses at this hearing, calling him out for how his “abuse of a young woman” perpetuates a “long history of exploitation.” David's response to her justified criticism is to “snap... back” at her, an aggravation not displayed toward anyone else on the committee [2]. In other words, David only lashes out in response to a woman who dares to speak her mind.

David's university hearing has often been considered a parallel to the Truth and Reconciliation Commission (TRC) [7]. The TRC was criticized “for advocating a form of the expression of

guilt expiation... [that] tended to perpetuate rather than to propitiate and absolve the sins of apartheid” [4]. The university seeking an apology from David for the sake of receiving an apology, uncaring if his remorse is genuine, is thus reminiscent of the TRC's strategy [2]. This assessment, while accurate, is incomplete, as it fails to consider how David's targeted rebuttals to Dr. Rassool's criticism suggest that the presence of a woman's protest negates any possibility of spiritual redemption for him; he cannot take women's opinions seriously. If we consider that Dr. Rassool may be a woman of color—her surname is of Arabic origin—then racialized misogyny must be brought into the conversation: David's adamant refusal to apologize manifests specifically in opposition to the informed outrage of a nonwhite woman, notably the first nonwhite woman in the narrative that he has not slept with. For David, then, sex appeal primarily constitutes his value of nonwhite women, not their ability to speak truth, hence his irritation toward Dr. Rassool's comments; her vocality does not conform to patriarchal, white supremacist standards that seek to silence women of color. Consequently, if racialized misogyny prevents nonwhite women's protest from encouraging spiritual growth, what *can* facilitate the spiritual element of David's redemption? We must look to the opposite end of the spectrum: the necessity of male patriarchal approval and the silencing of nonwhite women.

In the second half of the novel, David comes to understand the harm his behavior wrought on Melanie. To his credit, David's apology to Mr. Isaacs reads as sincere: “I am sorry for what I took your daughter through. You have a wonderful family. I apologize for the grief I have caused you and Mrs. Isaacs. I ask for your pardon” [2]. Upon closer inspection, however, David's apology foregrounds the reaction and reception of Mr. Isaacs, a man, in which the women involved are allowed no response. David specifically goes to Mr. Isaacs to offer his apology, and it is Mr. Isaacs alone who invites David into his home [2]. In the most literal sense, then, a man facilitates David's ability to deliver his apology. Additionally, David refers to Melanie as “your daughter” in the previous quote, emphasizing Mr. Isaacs' paternal authority as her father, whereas using her name would have prioritized her individual identity. David then asks for *Mr. Isaacs'* pardon, not Melanie's or even Mrs. Isaacs', again deferring to the patriarchal figure for recognition of his apology and thus the facilitation of his redemptive journey. The delivery of David's spiritual redemption therefore only occurs at the behest and the acceptance of a man, where the women involved, particularly three nonwhite women, cannot voice an opinion.

Scholars have previously acknowledged this silencing of nonwhite women in *Disgrace*. As media studies scholar Ian Glenn observes, “Women of colour seem destined to be without agency” in the novel, including if not especially in this spiritual turning point of David's redemptive journey [6]. Where David ignores the voice of Lucy, a white woman, the voices of Melanie, her mother, and her sister—implied to be women of color, perhaps South Africans of mixed race—are never given a chance to speak, especially regarding the changes in his character [5], [7]. We might further note that Melanie's mother and sister can only express their true feelings through body language: Desiree displays hesitance around David, and Mrs. Isaacs avoids his eyes [2]. This physical discomfort suggests that unlike Mr. Isaacs, these women might not be so keen as to accept David's apology in good faith. The women's display of nonverbal communication might evince how nonwhite women have

found ways to speak despite the suppression of their voices; there is no questioning the discomfort they feel around David. That said, their discomfort nonetheless goes unrecognized—or worse, ignored—by Mr. Isaacs. Patriarchy thus grants David's spiritual redemption through the disregard of nonwhite women's perspectives, from Dr. Rassool to the Isaacs' women, not unlike David's individual disregard for Lucy's autonomy. Even when David finally offers an apology to the Isaacs' mother and younger daughter, the narrative denies them an opportunity to reply. "With careful ceremony, [David] gets to his knees and touches his forehead to the floor" before Mrs. Isaacs and Desiree, where mother and daughter can only "sit... there, frozen" [2]. As nonwhite women, their silence in *Disgrace* reinforces the violent legacy of suppression of nonwhite women's voices, especially in opposition to whiteness and patriarchy. What's more, Melanie, David's victim, is notably absent from this apology; how can we consider the spiritual healing of David's redemption viable if he never attempts to offer his sincere remorse to the person he directly harms? As such, it is only through the racialized misogyny of nonwhite women's silence that David can achieve spiritual redemption. To consider the juxtaposition of Dr. Rassool's vocal criticism with the silence of the Isaacs' women, the implication is that only through women's silence, without a woman "nagging," was David's spiritual growth possible. Again, the unviability of *Disgrace*'s redemptive theory arises because of this "redemption's" suppression of nonwhite women's perspectives, in which the denial of their voices and autonomy becomes exploited for David's self-betterment.

### Personal Sacrifice and the Exploitation of Women's Pain

The final element of *Disgrace*'s redemptive theory is the necessity of sacrifice: "What if... what if *that* is the price one has to pay for staying on?" [2]. Though this quote refers to a specific moment of sexual violence, the concept can be construed to refer to suffering and sacrifice in general. What if suffering and sacrifice are the price oppressors must pay for their redemption? Many critics concede that the novel holds "an instinctive awareness of the need for... sacrifice as a basic condition of life in the new South Africa" [4]. As white South Africans, both David and Lucy embody this necessity of sacrifice because of their racial privilege; David committed an intentional act of sexual violence, while Lucy "by default... maintain[s] the traditional, defensive position of the white landowner in South Africa" [3]. However, in *Disgrace*, Lucy's suffering becomes instrumentalized to facilitate David's redemption, a strategy that is ultimately unviable because it posits redemption must require an unethical dependence on women's pain.

If David's journey is characterized by a "generalized regime of renunciation," then Lucy's is constructed through a dual regime of loss and additional burdens [3]. Much of David's sacrifice is imposed semi-willingly: he walks away from his job, moves out of his home, and in the final act of the novel, euthanizes the dog he connects with the most. The key suffering forced upon him is that of assault and physical mutilation, where the three attackers set him on fire and permanently damaged his ear [2]. Lucy, in contrast, is raped. Whereas David loses part of his ear, Lucy loses part of her vitality. She compares sex to murder—"When you [a man] have sex with someone... when you trap her, hold her down, get her under you, put all your weight on her – isn't it a bit like killing?"—and

ultimately concludes that after her rape, "[she is] a dead person and [she] do[es] not know yet what will bring [her] back to life" [2]. Where the suffering imposed on David only damages his pride, Lucy's suffering traumatizes her and leads to the reconstruction of her identity as someone who is dead—someone who has been murdered. David's redemptive journey then instrumentalizes her trauma: David only moves to a position of self-reflection because of "the parallels between [his] seduction-violation of Melanie and the rape of his daughter" [6]. For example, in the scene immediately after David's fruitless conversation with Lucy where he implores her to press charges for the rape and move out of her house, David returns to George to apologize to the Isaacs family [2]. This sudden desire for confession reflects an awareness of his own crime, his rape of Melanie, that has only developed after witnessing the mental deterioration of his daughter following her experience of sexual violence. Simply put, Lucy's pain allows David to recognize his own criminality, marking a step forward in his journey toward redemption.

Additionally, where semi-willingly "giving up" primarily constitutes David's sacrifice, semi-willingly "taking on" constitutes Lucy's. Upon learning her rapists have impregnated her, Lucy chooses not to get an abortion, instead vowing that she is "determined to be a good mother" [2]. As a result of this choice, Lucy takes on the additional burden of sacrificing years of her future in order to raise a child she never asked to bear. Although David initially questions Lucy's desire to keep the child, he comes to fixate on "[w]hat [it] will... entail [to be]... a grandfather," a future in which his grandchild can facilitate the development of new and better "virtues" for him. The product of violence against Lucy, the consequence of her suffering and sacrifice, is again instrumentalized to clear the path for David's redemption—he can become a "better" person because of the product of Lucy's rape [2]. Once more, patriarchy subtends the framework of *Disgrace*'s redemptive theory, where a woman's suffering facilitates a man's ability to grow. This overdependence thus reinforces the unviability of *Disgrace*'s theory of redemption—David is only "redeemed" through the exploitation of Lucy's victimization and sacrifice.

### Conclusion

The fact that patriarchy limits the generative possibility of *Disgrace*'s theory for redemption is unquestionable. What must remain contested, however, is whether the novel is aware of its dependence on patriarchal structures, or whether the very intent of *Disgrace* is to expose the unviability of a redemption that exploits the wisdom and suffering of women for male benefit. Perhaps the nuance with which the novel presents this redemption, such as the narrator's implicit critiques of David throughout, suggests the second is more likely. At the same time, does this criticism run the risk of replicating the patriarchal structures it seeks to expose? Either way, one can hardly challenge that *Disgrace* "offers a grim vision" of the future for South Africa, including if not especially the country's women [4]. And maybe for Coetzee to have written a more explicitly hopeful novel "would be to tell another kind of lie" because of this story's post-apartheid context—redemption and healing for all South Africans cannot come in a wink [1]. If nothing else, *Disgrace* reaffirms the futility of imagining a more just post-apartheid world when this world continues to perpetuate patriarchal systems that ensure the erasure, silencing, and suffering of women.



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# Prevalence of the Diffusion Collision Model of Protein Folding In Vivo: A Mechanistic Analysis of the Acceleration of Protein Folding by Peptidyl-Prolyl Isomerase and the GroEL/ES Chaperonin System

AATMAANANDA NAGAR NAYAK  
IMPERIAL COLLEGE OF LONDON

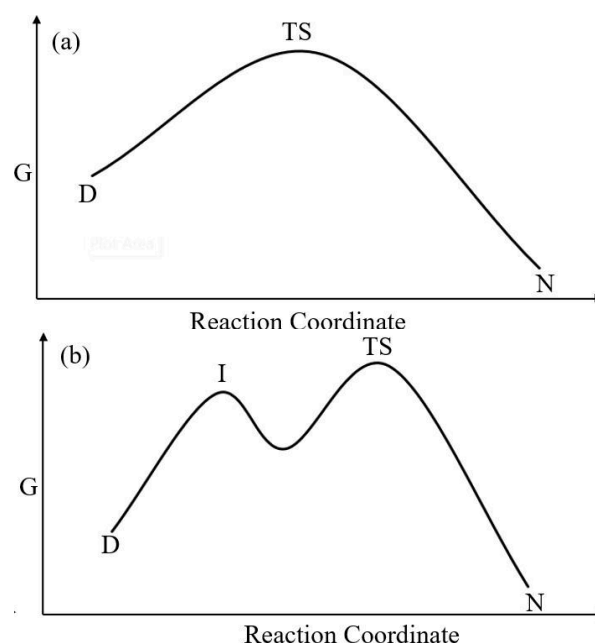
This review assesses the differences between the diffusion-collision model and the extended nucleation condensation model of protein folding and attempts to determine; by analyzing the mechanisms through which peptidyl-prolyl isomerase and the GroEL/ES chaperonin system accelerate the rate of folding of their respective substrate proteins, which model of protein folding prevails *in vivo*. The difference in the kinetics between the two protein folding models was assessed in the introduction using free energy profiles which led to the identification of conditions that would favour one model over the other. Following the justification of the choice of chaperones used for analysis, the mechanism through which both chaperones accelerated the rate of folding of their respective proteins was investigated to determine whether the conditions developed by the chaperones were consistent with one model of protein folding over another. The review concludes with a summary of the key findings gleaned from mechanistic analysis of chaperone function and highlights its relevance to the biochemical and medical fields.

**Keywords:** Protein Folding, DCM, ENCM, PPIase, GroEL/ES, In Vivo, Unfolded State, Native State

## Introduction

The kinetic and thermodynamic features of the protein folding process have been a matter of intense study amongst biochemists and biophysicists for the better part of a century. Yet much remains to be known about the process through which proteins 'fold' (renature) from an 'unfolded' (denatured) polypeptide chain into their 'native' (functionally active) state.

Proteins have been theorized to fold via one of two mechanisms that, in essence, differ in their transition states and free energy profiles: the diffusion collision model (DCM) and the extended nucleation condensation model (ENCM) [1]. This paper suggests that the DCM is the prevalent protein folding process *in vivo*. The DCM postulates that segments of the unfolded polypeptide, via local interactions, fold rapidly to form secondary structure elements, such as  $\alpha$ -helices and  $\beta$ -sheets, in a non-rate-determining step. These marginally stable secondary structure elements, referred to as microdomains, then coalesce in a diffusion-controlled manner to form the native state, stabilized by tertiary interactions that form when the microdomains interact. The topology of this transition state does not necessarily resemble that of the native state. [2]. The ENCM, on the other hand, posits that both local and long-range interactions concomitantly contribute to the formation of a weak, extended nucleus, to form a transition state with a topology resembling the native state [3]. The difference in free energy profiles between both models is shown in Fig 1.



**Figure 1:** Free energy profiles for the ENCM and the DCM of the protein folding pathway from the unfolded, denatured state (D) to the native state (N). (a) displays the

free energy profile for the ENCM wherein secondary and tertiary interactions form concomitantly to form the transition state (TS). (b) displays the free energy profile for the DCM wherein secondary interactions form before tertiary interactions to produce intermediates (I) in a non-rate-limiting step preceding the formation of the TS.

Molecular chaperones (referred to as only 'chaperones' in the rest of the text) are a family of proteins that assist in the folding of polypeptide chains into their native state, without being a part of that final native state [4]. Studies of the interactions between a chaperone and its substrate protein, as the protein folds, have provided insights into the mechanisms employed by the chaperone to accelerate the folding process. Examining these mechanisms could provide an insight into whether the DCM's or the ENCM's transition state and intermediates are preferentially stabilized by chaperones. The large energetic expenditure made by cells in maintaining an intricate network of chaperones indicates that they are vital to maintaining the cellular proteome, via control of protein folding [4]. Studying the mechanisms through which some of these chaperones accelerate protein folding in cellular environments may provide insights as to whether the DCM or the ENCM prevails *in vivo*. Exploring the conditions that would stabilize such species for both models would provide a starting point for such an analysis.

As stated before, in the DCM the protein folds into its native state through the collision of individual microdomains (secondary structure elements that form rapidly due to local interactions) in the 'correct' (native) orientation, which contributes to the formation of long-range tertiary interactions that stabilize the microdomains in their native orientation. The rate-limiting step is not the formation of the microdomains but rather their diffusion into the 'correct' orientation, making it a diffusion-controlled process. Hence, the rate of this process is inversely proportional to the viscosity ( $\eta$ ) of the solution [5]. Furthermore, the folding pathway of the DCM is populated with multiple intermediates that may differ in topology (due to the absence of tertiary interactions in the first step of the process) but are relatively similar in their free energy contents (G). Since microdomains form rapidly, they would have similar levels of secondary structure which implies similar free energy content [6] between intermediates. In the ENCM, however, the formation of an extended nucleus, that precedes the transition state, requires highly specific secondary and tertiary interactions. As a result, the number of initial conformations that can fold into an extended nucleus is minimized, resulting in minimal intermediates present in the ENCM folding pathway [3]. A decrease in viscosity and/or an increase in the rate of diffusion of microdomains would not increase the rate of formation of the transition state in the ENCM.

### Choice of Chaperones for Analysis

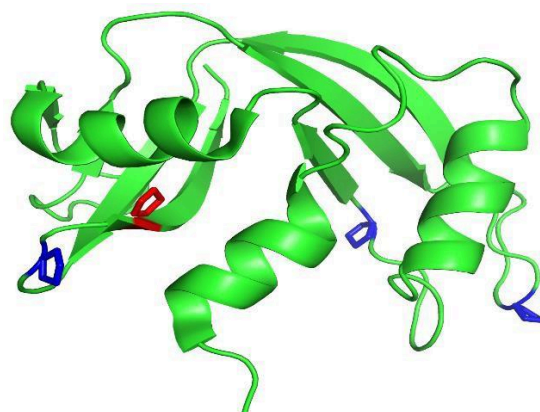
The interactions between peptidyl-prolyl isomerases (PPIases), the GroEL/ES chaperonin system, and their respective substrates were chosen for analysis based on their employed mechanism of accelerating the folding process: PPIases accelerate protein folding via chemical means (by catalyzing peptidyl-prolyl isomerization) [7] whereas the GroEL/ES chaperonin system relies primarily on mechanical means to achieve the same effect [8]. This ensures that both the biophysical and chemical factors of the protein folding

process are considered while determining whether the DCM or the ENCM is the prevalent model for protein folding *in vivo*.

### PPIase

PPIases serve an essential role in the folding pathway of proline-containing polypeptides [9]. Amino acids in polypeptide chains are linked by peptide bonds, which have a partial double bond character. This introduces rigidity into the protein backbone as rotation about the carbon-nitrogen peptide bond is highly restricted, allowing it to exist only in either the cis or the trans conformation. The height of the energetic barrier to rotation about the double bond to allow for cis/trans isomerization is influenced by the properties of the side chains of the amino acids in the peptide bond [10]. The rotational barrier about X-Pro bonds (where X is any amino acid) serves as a kinetic hurdle in the protein folding process [11]. Interactions between PPIases and their respective substrates could provide greater insight into the mechanism of protein folding. Interactions between denatured bovine pancreatic ribonuclease A (RNase A) and PPIase isolated from pig kidney were analyzed to determine the mechanism by which RNase A folding was accelerated.

RNase A is a 14 kDa protein that contains four X-Pro bonds within its primary structure [12]. PPIase-mediated acceleration of RNase A folding is thought to proceed via catalysis of cis/trans isomerization about the X-Pro bonds. [13]. The location of X-Pro sites on RNase A reveals the potential sites of interaction between RNase A and the PPIase isolated from pig kidney, providing insight into the mechanism through which protein folding occurs.



**Figure 2:** Cartoon representation of the bovine pancreatic ribonuclease A crystal structure (PDB code 1KF5) with only proline side chains visible and highlighted; blue indicates the presence of the X-Pro bond on a turn/loop whereas red indicates the presence of the X-Pro bond on a secondary structure element (microdomain). PyMol was used to make this figure.

From Fig 2., it is evident that of the four X-Pro sites on RNase A, three are present in turns and loops that connect the more regular and ordered secondary structure elements (microdomains). Only one X-Pro site exists within a microdomain (the X-Pro bond containing Pro117). Thus, 75% of the X-Pro sites are present on loops and turns that serve to connect microdomains, rather than stabilize their structure. Though the loops and turns do not possess a

regular, repeating structure they are not the same as a random coil and have a defined structure in the native state [14]. Catalysis of cis/trans isomerization about the X-Pro bond can be shown to increase the proportion of collisions between microdomains in the 'correct' orientation to form the long-range interactions needed to form and stabilize the native tertiary structure. The first equation that needs to be consulted is the Stokes-Einstein equation (SE equation) which allows for the calculation of the diffusion coefficient (D) as shown below.

$$(1) \quad D = \frac{k_B T}{3\pi\eta d}$$

In equation (1),  $k_B$  is the Boltzmann constant,  $T$  is the temperature in kelvin,  $\eta$  is the viscosity, and  $d$  is the diameter of the considered particle (its shape is approximated to a sphere) [15]. Given that these parameters are unaffected when RNase A folding is accelerated by PPIase [10] the value of  $D$  remains the same. To understand the influence of the PPIase on accelerating the folding of RNase A, Fick's first law of diffusion needs to be considered.

$$(2) \quad J = -D \frac{\partial C}{\partial x}$$

In equation (2), which is Fick's first law,  $J$  is the flux (amount of matter passing through the point  $x$ ),  $C$  is the concentration at  $x$ , and  $x$  is the given position [16]. The minus sign indicates that the flow of matter occurs from a higher concentration to a lower concentration. This can be equated to protein folding by considering concentration in terms of free energy, wherein movement across a steeper concentration gradient equates to a larger change in free energy [17]. Moving from a region with a higher concentration to a region with a lower concentration is correlated to a decrease in free energy. According to the energy landscape theory, the native state of the protein represents the global free energy minimum of the folding funnel [18]. Conformational and configurational changes to RNase A structure, such as the cis/trans isomerization of X-Pro bonds catalysed by the PPIase, move it further along the folding funnel, away from its unfolded random coil state and closer towards its native state, which correlates to a decrease in free energy. This equates to movement down the concentration gradient, towards the lower concentration, which increases the value of  $J$ . This indicates that accelerating the folding of RNase A from its unfolded to its native state requires an increase in the amount of flux, which resonates with a diffusion-controlled process such as the DCM over the ENCM. Kramer's theory of diffusion over a potential energy barrier, represented by equation (3) [16], provides further support for the DCM over the ENCM.

$$(3) \quad J = D \left( -\frac{p}{k_B T} \times \frac{dU}{d\xi} - \frac{dp}{d\xi} \right)$$

In equation (3), the variables  $J$ ,  $D$ ,  $k_B$ , and  $T$  represent the same parameters as in equations (1) and (2) while  $U$  represents potential energy,  $p$  represents the probability of a molecule occupying a specific position along a reaction coordinate, and  $\xi$  represents a length element (extrapolated in the equation below, where  $t$  represents the time interval of a particle with mass  $m$  to move across a given position).

$$(4) \quad \xi = t \sqrt{\frac{k_B T}{m}}$$

Equation (3) is of interest, however, as it replaces the concentration gradient, from equation (2), with a probability gradient while introducing a value  $-U$  to represent the potential energy barrier imposed onto the RNase A folding process by the need for cis/trans isomerization. The PPIase would serve to increase the flux of microdomains ( $J$ ) over potential energy barriers (towards the transition state) by minimizing the height of the barriers through catalysis of cis/trans isomerization about the X-Pro bonds. This aligns more with the DCM than with the ENCM because (as highlighted earlier) an increase in the rate of collisions between microdomains favours a diffusion-controlled process over a process that requires the simultaneous formation of specific local and long-range interactions to form an extended nucleus. Furthermore, the nature of the DCM implies the presence of multiple intermediates preceding the transition state that possess a similar free energy content despite differing in their overall topology (as described earlier). This would imply that the probability of a given RNase A molecule occupying a specific position immediately preceding the transition state would be greater in the DCM than in the ENCM since the formation of an extended nucleus minimizes the number of intermediates formed (as described earlier) [3]. Thus, the value of the probability gradient in equation (3) would be greater in the DCM than in the ENCM. As a result, even if an increase in the flux of microdomains could accelerate RNase A folding through the ENCM, the larger value of the probability gradient in the DCM (due to the presence of intermediates) would render it a more feasible pathway for accelerated RNase A folding (following interaction with the PPIase). Thus, interactions between the PPIase and denatured RNase A that serve to accelerate its folding into the native state provide greater support for the DCM than the ENCM as the protein folding pathway is accelerated by PPIase.

### GroEL/ES Chaperonin System

The GroEL/ES chaperonin system (referred to as 'GroEL/ES' in the rest of the text) is comprised of the GroEL chaperone – which consists of two 57 kDa heptameric rings that form a cylindrical structure with two cavities and an ATPase domain – and the GroES co-chaperone – which consists of a 10 kDa heptameric ring that binds to the end of the GroEL cylinder via an ATPase cycle that triggers structural changes in the GroEL chaperone [19]. Since GroEL/ES forms nano-cages that assist in and accelerate the folding of various polypeptides of differing sizes that do not possess an obvious common property (as opposed to the substrates of PPIases wherein they all possess X-Pro bonds) [8], studies detailing the kinetic data of the accelerated folding of various proteins associated with GroEL/ES were consulted for analysis. These proteins were ribulose-1,5-bisphosphate-carboxylase-oxygenase (RuBisCo), dihydrodipicolinate synthase (DapA), malate synthase G (MSG), citrate synthase (CS), and rhodanese. Under permissive folding conditions (wherein spontaneous protein folding can proceed unhindered and off-pathway aggregation of proteins is avoided) the presence of GroEL/ES accelerated the rate of protein folding for every protein listed above, except for rhodanese [8, 19, 20, 21, 22]. A review of the different models proposed for the mechanism of



function of GroEL/ES could provide insights into why that may be and could indicate which model of protein folding is best suited to the chaperone-mediated acceleration of the folding process.

It has been posited that the enclosed cavities within GroEL/ES (that arise following ATP-dependent binding) form nano-cages that can assist in protein folding through either the passive cage model (sometimes referred to as the 'Anfinsen cage' model), the active-cage model, or the iterative annealing model [8]. The passive cage model suggests that the nano-cage serves to infinitely dilute the substrate protein from other macromolecules to prevent aggregation [23]. In this model, there is no specific mechanism employed to accelerate the protein folding process; it only functions to provide permissive conditions for protein folding and, therefore, does not provide any insight into the kinetics of the protein folding process. The other two proposed models, however, are of interest as they posit that the changes that occur to the structure of the GroEL chaperone following ATP-dependent GroES binding accelerate the rate of protein folding and, thus, may provide insight into the kinetics of the protein folding process.

The active-cage model suggests that confinement within GroEL/ES serves to accelerate protein folding (beyond prevention of off-pathway aggregation) on the basis that ATP-dependent GroES binding to GroEL (following substrate protein binding) triggers several changes: a small increase in the size of the cavity and an increase in the polarity of the cavity lining, as hydrophobic residues are buried to leave a hydrophilic cavity lining [24]. Both changes serve to increase the flux of microdomains within the solution present in the GroEL/ES cavity (referred to in the rest of the text as 'cage solution'), which would be expected to accelerate the rate of protein folding via the DCM, as explained previously. The small increase in the volume of the cavity (while the number of water and protein molecules present in the cavity remains constant) results in a decrease in pressure, according to equation (5).

$$(5) \quad P = \frac{nRT}{V}$$

In equation (5),  $P$  is the pressure,  $n$  is the number of moles of solution,  $R$  is the gas constant,  $T$  is the temperature (in Kelvin), and  $V$  is the volume of the container. Since  $n$ ,  $R$ , and  $T$  remain constant in the GroEL/ES cavity, the increase in volume produces a decrease in pressure. At lower pressures, liquids display a lower viscosity [25] which, as established earlier in relation to equation 1, corresponds to an increase in the diffusion coefficient. An increase in the rate of diffusion would accelerate a diffusion-controlled reaction such as the DCM, providing further support for its prevalence *in vivo*. The change in polar character of the GroEL/ES cavity lining from hydrophobic to hydrophilic also contributes to a decrease in the viscosity of the cage solution within the GroEL/ES cavity. Transient polar interactions between the microdomains of partially folded intermediates of the substrate protein in the GroEL/ES cavity and the hydrophilic cavity lining serve to (temporarily) decrease the effective concentration of microdomains in the cage solution available for collision. According to the DCM, microdomains can be considered independent units whose interactions with each other to form the native protein rely on random diffusion. Thus, these microdomains can be viewed as independent solute molecules in the cage solution, and (temporarily) decreasing their concentration (via transient polar interactions with the hydrophilic cavity lining) would

lead to a drop in the viscosity of the cage solution [26]. The resulting increase in the rate of diffusion in the cage solution would lead to an accelerated rate of protein folding only through the DCM and not through the ENCM, as explained earlier. The use of the DCM as the prevalent model of protein folding *in vivo* may also explain why only the rate of folding of rhodanese is not accelerated by the active-cage model (beyond prevention of off-pathway aggregation).

The hydrophilic lining of the GroEL/ES cavity (following substrate protein binding and an ATPase cycle) can interact with polar water molecules and sequester them from the cage solution. This would decrease the number of water molecules available in the cage solution to interact with the microdomains of the substrate protein to form nonnative protein-water interactions. This drives a decrease in the number of intermediates of the substrate protein that form nonnative protein-water interactions (instead of native protein-protein interactions for example). Partition functions ( $Q$ ) can be used to group and differentiate between sets of microstates (intermediates of the substrate protein) that correspond to a specific internal energy and conformational entropy. The potential energy and entropy terms can be summed up and shown using free energy and so a partition function can be defined as a sum over a set of microstates that possess the same free energy, as shown in equation (6).

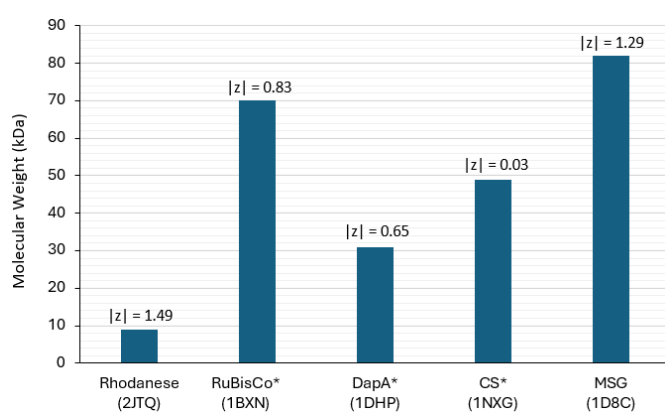
$$(6) \quad Q = \sum_i^{nts} e^{-E_i/k_B T}$$

In equation (6),  $nts$  is the total number of microstates with a specific free energy,  $E_i$  is the free energy of a microstate with index  $i$ , and  $k_B$  and  $T$  are the same values as shown in equation (1). Sequestration of water molecules from the cage solution by the hydrophilic lining of the GroEL/ES cavity serves to decrease the number of microstates wherein nonnative protein-water interactions form. This decreases the value of the partition function that represents the sum of microstates in which these nonnative protein-water interactions are formed with the same free energy (represented by  $Q_{pw}$ ). A similar partition function can be used to represent the sum of microstates in which native protein-protein interactions are formed with the same free energy (represented by  $Q_{pp}$ ).  $Q_{pp}$  comprises a set of intermediates that are not kinetically trapped and have the potential to form the transition state (as their free energy corresponds to that specific position along a reaction coordinate) and a simplified equation can be used to model the probability of such an intermediate molecule occupying a specific position along a reaction coordinate ( $p_{pp}$ ), as shown in equation (7).

$$(7) \quad p_{pp} = \frac{Q_{pp}}{Q_{pp} + Q_{rs}}$$

In equation (7),  $Q_{rs}$  represents the sum of the remaining microstates not encompassed by  $Q_{pp}$ .  $Q_{pw}$  is a subset of  $Q_{rs}$ , and the hydrophilic lining of the GroEL/ES cavity would serve to decrease the value of  $Q_{pw}$  and, thus,  $Q_{rs}$ . This results in a decrease in the value of the denominator in equation (7) and an increase in the value of  $p_{pp}$ . As seen in equation (3) increasing the probability of finding a molecule at a specific position along a reaction coordinate would drive an increase in the flux of microdomains ( $J$ ) that (as explained previously in the text) is consistent with an acceleration in the rate of protein folding through the DCM rather than the ENCM. However, such an increase in the flux of domains is reliant

on the value of  $Q_{pw}$  decreasing significantly following the sequestration of water molecules from the cage solution by the hydrophilic cavity lining of GroEL/ES. Rhodanese is an outlier amongst RuBisCo, DapA, MSG, and CS in terms of molecular weight, as can be seen in Fig 3. It should be noted that for multimeric proteins (RuBisCo, DapA, and CS) the molecular weights of their independent monomeric subunits were compared as research suggests that individual subunits are folded separately within chaperone cages before complex assembly to form the multimeric protein [27].



**Figure 3:** Comparison of the molecular weights (in kDa) of the five proteins whose rates of folding were measured in permissive conditions in the presence and absence of GroEL/ES. The presence of an asterisk (\*) next to the name of the protein indicates that it is multimeric and that the molecular weight of its polypeptide monomer was used for comparison. The respective PDB codes of each protein are shown in brackets below its name and the modulus of the  $z$  value of the molecular weight of each protein (or its subunit) is indicated above its respective bar (as  $|z|$ ). The modulus of the  $z$  value indicates the magnitude of the difference (in terms of the number of standard deviations) between the protein's molecular weight and the mean molecular weight of the five proteins.

From the figure above it is evident that rhodanese is the outlier, in terms of molecular weight, amongst the five proteins, as it has the largest absolute  $z$  value. This could indicate that its low molecular weight (9.44 kDa) may play a role in GroEL/ES not being able to accelerate the rate of folding of rhodanese (beyond providing permissive folding conditions). If protein folding within the GroEL/ES cavity were to proceed via the DCM, an acceleration of the process would require a significant decrease in the value of  $Q_{pw}$  for that protein as posited in equation (7). For a substrate protein as small as rhodanese, the absolute number of nonnative protein-water interactions would be significantly smaller compared to a protein with a larger molecular weight, on the basis that there would be fewer microdomains present (in rhodanese's structure) to form such protein-water interactions. As a result, the number of water molecules sequestered from the cage solution by the hydrophilic cavity lining of GroEL/ES may not be enough to contribute to a decrease in the value of  $Q_{pw}$  for rhodanese as there would likely be

enough water molecules left in the cage solution to contribute to the formation of rhodanese microstates with nonnative protein-water interactions. The inability of GroEL/ES to accelerate the folding of rhodanese (beyond preventing aggregation) due to an inability to increase the flux of rhodanese microdomains indicates that protein folding occurs within the GroEL/ES cavity via the DCM rather than the ENCM, in the active-cage model of chaperonin function.

The iterative annealing model of chaperonin function suggests that repeated binding and release of partially folded intermediates of the substrate protein accelerates its folding toward the native state [28]. This model posits that along the folding pathway of the substrate protein, there exist intermediates that possess nonnative protein-solvent interactions and nonnative protein-protein tertiary interactions that serve as kinetic traps that do not contribute to productive protein folding [29, 30]. In this model, GroEL/ES binds to such misfolded intermediates (in an ATP-dependent manner) and triggers partial unfolding of the intermediate (wherein nonnative interactions are disrupted) before subsequent release into solution to allow the appropriate native interactions to form [31]. The disruption of nonnative interactions in intermediates of the substrate protein by GroEL/ES [27] serves to decrease the height of the potential energy barrier ( $U$ ) in equation (3) which would drive an increase in the flux of microdomains. Thus, the iterative annealing model suggests the presence of multiple intermediates of the substrate protein and suggests that the GroEL/ES acts to increase the flux of microdomains into the 'correct' orientation to drive the formation of native tertiary interactions to stabilize the native state. These are features of the protein folding process that are implied by the DCM which indicates that the iterative annealing model of chaperonin function provides further support for the DCM prevailing as the GroEL/ES-mediated protein folding pathway.

Thus, both the active-cage model and the iterative annealing model of GroEL/ES function support the idea that the DCM, rather than the ENCM, is the pathway through which protein folding is accelerated by GroEL/ES.

## Conclusion

From this review of the acceleration of protein folding by PPIases and GroEL/ES, using statistical mechanics, it can be concluded that the folding process exhibits kinetic and thermodynamic features that indicate that it proceeds via the DCM rather than the ENCM. As has been established, PPIases and GroEL/ES accelerate protein folding essentially through an increase in the flux of microdomains which is consistent with the DCM. Owing to the presence of the vast, intricate network of chaperones that maintain the proteome within cells, these findings imply that the prevalent model of protein folding *in vivo* is the DCM.

However, it must be noted that the DCM and the ENCM are both extremes of a process in which the transition state possesses secondary and tertiary interactions with the difference being the sequence in which those interactions are formed (which shapes the kinetic profile of the process) [2]. A better understanding of the kinetics associated with protein folding *in vivo* may be crucial in developing drugs and treatments for diseases associated with protein misfolding and aggregation. An example is Alzheimer's disease wherein PPIases and their cellular roles are being investigated to gain a deeper insight into the mechanism of tau protein aggregation and

the development of potential therapeutics [7].

Beyond the discussion that debates the acceptance of the DCM and the ENCM as the prevalent model of protein folding there persist debates between other ideas, such as the foldon-dependent hypothesis and the energy landscape theory [32, 33], that present further questions regarding the protein folding pathway. Further research and analysis of such models may perhaps allow for the development of a universal model of protein folding (that may borrow elements from each of these models) and would allow for the accurate prediction of a protein's tertiary structure from its primary structure, based on first principles alone.

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# The Fabrication of Colored Cellulose-Based Hydrogels for Solar Water Purification

**JOWON (LAYLA) CYHN**  
*DARTMOUTH COLLEGE*

Pollution and the depletion of clean drinking water sources have made maintaining freshwater supplies a subject of constant concern. Industrial waste dumping within aquatic ecosystems has further compounded this problem and contributed to a rise in infectious waterborne diseases, such as botulism and cholera (Schooner 2015). Therefore, environmentally responsible water purification technology development is essential to ensure a reliable supply of fresh, potable water. One of the most promising emerging technologies for water purification is the hydrogel. Recent studies have shown that hydrogels lower the amount of solar energy needed to evaporate water, thus increasing the efficiency of evaporation-based solar water purification systems (Weerasundara et al. 2021). Although evaporation efficiency rates may vary with the structure and composition of the hydrogel, no study has yet ascertained how hydrogel color affects evaporation efficiency rates. Therefore, in this study, I investigated the impact of different hydrogel colors on water evaporation rates in an experimental solar water purification system utilizing xenon light. The hydrogel colors tested included red, green, and translucent. Consistent with the assumption that hydrogels with the darkest dye would promote the fastest evaporation, red hydrogels performed the fastest, followed by green and transparent hues. This effect may be due to different dyes absorbing different wavelengths of light at unequal rates. Overall, this study provides new insights into the future direction of hydrogels and can help address remaining challenges in the field of water purification.

**Keywords:** Water Purification, Urbanization, Hydrogels, Pollutants, Potability

## Introduction

Amongst the extensive range of negative effects caused by anthropogenic environmental degradation, water pollution is amongst the most urgent (Schnoor, 2015). Several countries have already installed next-generation water purification technologies, such as desalinators, acoustic nanotubes, and photocatalytic systems (Weerasundara et al., 2020). Despite these efforts, next-generation water purification systems are expensive, hard to maintain, and require large amounts of energy, which makes them unfeasible for lower-income communities (Hong et al., 2020).

As an alternative to these overly expensive purification technologies, the hydrogel—a cross-linked hydrophilic polymer with a durable, three-dimensional structure—has gained increasing interest for its affordability and seemingly unlimited potential in purification systems (Xu et al., 2023).

Scientists have incorporated hydrogels into water purification systems to identify key hydrogel properties that can enhance the evaporation process. For example, hydrogel size, composition, shape, and color can all affect the water evaporation rate by affecting the way the hydrogel absorbs light and heat or interacts with particulate matter and water molecules (An et al., 2022). The most commonly studied hydrogels are synthetic hydrogels, which have been manufactured from scratch in a laboratory setting, and hybrid hydrogels, which share features of both natural and artificial hydrogels (Chamkouri et al., 2021).

Cellulose-based hydrogels are particularly appealing because they are biodegradable (cellulose is found in the cell walls of green plants, algae, and oomycetes) and highly absorbent, making them ideal for water purification applications (Cao et al., 2022).

Overall, hydrogels have demonstrated enormous potential across the fields of biology, chemistry, and physics, but the vast majority of research has focused on an insufficient range of hydrogel types (van Tran et al., 2018). Because hydrogels remain largely unexplored, scientists are still discovering the full potential of these unique substances, but further research is urgent. By exploring systems in which hydrogels serve as catalysts to accelerate evaporation, scientists can further develop a sustainable alternative to existing water treatment technology and produce a potentially invaluable tool for maintaining access to the vital ingredient of life—clean, potable water. The results of this research have the potential to support future breakthroughs in sustainable, hydrogel-assisted solar water purification technology, with the hope that these technologies can help achieve the United Nations' Sustainable Development Goal of ensuring access to clean water and sanitation for all. Further research could focus on optimizing the composition, structure, and color of cellulose-based hydrogels to improve their efficiency in purification systems. Additional studies should test how other environmental factors, including humidity, room temperature, and solar radiation, may affect the efficiency of hydrogel-based water treatment.

## Literature Review

### *Modern water purification and its problems*

It is crucial to take into account previous studies on hydrogels in order to comprehend the distinctive contribution of this study. To begin with, one such study states that the critical issue of a deficit in clean water and basic sanitation is likely to become even more pronounced in the coming years due to population growth, climate change, and urbanization (Shannon et al., 2008). Therefore, to address these issues, scientists and engineers are developing innovative technologies and methods for water purification, desalination, and wastewater treatment. Building on this paper, another related study states the main goal of water purification research revolves around improving water disinfection and decontamination. The authors then go on to explain that traditional methods such as chlorination and ozonation can be effective, but can also create harmful byproducts and have high energy requirements (Mueller et al., 2007). In that same vein, it is also recorded that researchers, in the past, have surveyed disinfection byproducts in the United States and found that many disinfection byproducts are generated during the disinfection process (Lantagne et al., 2006), effectively proving that there are several hazards associated with current systems.

While many past studies emphasize developing new technologies for water treatment, their focus remains on developing the existence of such systems rather than ways to accelerate evaporation. Additionally, a majority of this research focuses on expensive and high-energy consumption technology. Therefore, this study differs from past studies considering it uses an accessible, transferable, and biodegradable substance. Inclusive and improved clean water access and sanitation provide immeasurable benefits to human society, including public health, economic development, and sustainability.

### *What is a hydrogel?*

The term 'hydrogel' encapsulates a group of hydrophilic polymers that can hold or absorb a large amount of water (Nie et al., 2020). Hydrogels can be produced from either natural or synthetic materials, although synthetic hydrogels have become increasingly popular because of their superior water absorption capacity and longer lifespan (Ahmad et al., 2022). Additionally, the raw materials needed to produce synthetic hydrogels are also more readily available than natural materials—therefore, due to their unique physicochemical properties, hydrogel-based products are now widely involved in various industrial and environmental applications (Jayakumar et al., 2020). Nevertheless, these studies fail to examine the physical characteristics that deter hydrogel's ability to purify. This paper will thereby address this gap in existing literature by exploring changes in external color impact of water purification.

### *Advantages of cellulose-based hydrogels*

Cellulose-based hydrogels offer several benefits over conventional hydrogels. Among some of these advantages include (but are not limited to) them being biodegradable, biocompatible, and made from readily available raw materials (cellulose) (Kabir et al., 2018). Cellulose-based hydrogels also have good mechanical strength, high water-holding capacity, and can be easily modified to control their properties.

### *Water purification using hydrogels*

The polymeric networks in a hydrogel can be tailored to regulate the water state and incorporate solar absorbers, making hydrogels a promising platform for efficient solar water purification. Hydrogel-based water purification has the potential to demonstrate improved performance, scalability, stability, and sustainability relative to traditional water treatment systems. For example, Zhao et al. fabricated a floatable composite hydrogel using squid ink nanoparticles, silica aerogel, poly(vinyl alcohol), and acrylamide. This hydrogel was then used to increase the efficiency of solar evaporation-based desalination. Yu et al. showed that their experimental solar water purification system, based on a hybrid hydrogel evaporator, effectively removed heavy metal ions from contaminated water. Their system operated with a high evaporation rate and exhibited a natural anti-salt-fouling function, making it a promising solution for sustainable solar-driven water purification systems.

### *Solar water purification using differently colored, cellulose-based hydrogels*

Despite these many advances in the use of hydrogels for water purification, few studies have focused on *how* hydrogels absorb the solar energy necessary for solar-powered water purification or how this process could be further optimized. I thereby synthesized cellulose-based hydrogels and used watercolor dyes to color them. By testing these colored hydrogels in an experimental solar water purification system, I was able to experimentally determine which hydrogel color is most efficient for solar water purification.

### *Comparative analysis of double-degradable hydrogels and cellulose-based hydrogels*

Numerous experiments have investigated potential hydrogel features that make it appropriate for environmental applications. As reported by Zhang et al. (2020), a novel double-degradable hydrogel synthesized using yeast, polyvinyl alcohol (PVA), and carboxymethyl cellulose (CMC) tends to have both excellent physical and mechanical properties that make it suitable for environmental application. More specifically, per the research findings, these hydrogels exhibited superior biodegradability and swelling capabilities, making it an appropriate reagent for water purification.

Zhang et al. (2020) developed double-degradable hydrogel through the freeze-thaw method. Mc Gann (2009) defines freeze-thaw as the freezing and thawing of a hydrogel solution at room temperature, which results in the creation of ice that produces a void inside the structure of the hydrogel, whereas thawing causes the ice crystals to collapse. A porous hydrogel will be generated when this procedure is repeated numerous times. Additionally, using scanning electron microscopy (SEM) and tensile strength testing, Zhang et al. (2020) categorized the double-degradable hydrogel's physical and mechanical properties. The thermal stability of a novel double-degradable hydrogel was determined using thermogravimetric analysis (TGA) which, according to Kubiski et al. (2023), is a procedure that involves weighing a hydrogel while it is heated and cooled in an experimental environment. The study reveals that the weight of a hydrogel with great thermal properties will grow as its temperature rises and decrease as it cools, primarily as

a result of the release of volatile components. In contrast to my study, in which the hydrogel was manufactured using cellulose biopolymer and the Zhang et al. (2022) production approach. For the evaluation of the optical and thermal properties of the synthesized cellulose-based hydrogel, I applied watercolor dyes to the hydrogel. This step would assist in determining the ideal hydrogel concentration for the water purification process while exposed to solar radiation. The evaporation rate of the synthesized hydrogel was then determined by measuring the rate of water evaporation under a xenon lamp every six hours for 24 hours to determine the water-retention capacity of cellulose-based hydrogel compounds.

Even though Zhang et al. (2020) study on the application of hydrogels in water purification yielded encouraging findings, further research is required. First, Zhang et al. (2020) did not investigate the level of toxicity of double-degradable hydrogel when discarded into the environment. My research addresses this issue by manufacturing a hydrogel composed of cellulose, a naturally occurring polymer that does not emit any type of hazardous substance into the environment. According to Sannino et al. (2009), not only is the freeze-thaw procedure time-consuming, but polyvinyl alcohol (PVA) is also not a naturally occurring substance, unlike cellulose, which is abundant and cost-effective in large-scale implementation.

#### *The optimization of hydrogel matrices' design and performance for solar-powered water purification*

The increased interest in hydrogels for solar-powered water purification is attributable to their high water absorption capacity and elastic properties. Researchers Guo and Yu (2021) delved into the synthesis of solar water purification systems utilizing hydrogels. Their study focused on thermal properties and how they can be optimized to enhance hydrogels' solar energy absorption and heat transfer rate for a more efficient water purification system. However, in contrast to my study, Guo and Yu's (2021) research centered on optimizing the hydrogel structure and structure for a solar water purification system.

In their study, Guo and Yu (2021) employed various methods to increase the solar absorption of hydrogels, such as the incorporation of light-absorbing materials like carbon black, graphene, and metal nanoparticles into the hydrogel structure. By increasing the light absorption properties of the hydrogel, the rate of solar-driven water evaporation will also improve. This can be achieved by incorporating carbon black and graphene into the Hydrogel matrices. According to the study, including silver and gold nanoparticles can improve hydrogel matrices' solar absorption and antimicrobial properties. In addition, Guo and Yu (2021) found that combining light-absorbing materials into hydrogels improves their swelling and mechanical properties. These characteristics are essential to hydrogel matrices because they preserve the hydrogel's structural integrity and water absorption capacity during solar desalination and water purification. Zhang et al. (2022) show that cross-linking density and chemical composition can enhance hydrogel's swelling and mechanical properties. In addition, the study demonstrates that the material's swelling and mechanical properties can be optimized by using a suitable hydrogel matrix. For example, synthesizing hydrogel based on the research of Song et al. (2023) can improve its swelling properties and biocompatibility.

In contrast, my research aims to investigate hydrogels' synthesis and potential applications in solar-powered water purification systems. As previously mentioned, Guo and Yu's (2021) research aimed to optimize the structure of hydrogels by improving their thermal and optical properties. Even though the study presented an efficient hydrogel for water desalination and purification, it needed to fully account for the solar energy absorption required for the purification process. The study conducted by Guo and Yu (2021) focuses on using graphene and carbon nanotubes to optimize the structure of their hydrogels. Nevertheless, Xiao et al. (2023) study reveals several drawbacks to incorporating these light-absorbing materials into hydrogel matrices. Carbon black harms aquatic organisms, which is a significant disadvantage. Similarly, gold and silver used as metal nanoparticles in optimizing the hydrogel matrices can have toxic effects on marine and terrestrial organisms and contribute to the accumulation of toxic metals in the environment.

Moreover, as shown by Xiao et al. (2023), incorporating these materials into research affects the mechanical properties of the hydrogel. For instance, incorporating gold nanoparticles into a hydrogel structure can reduce its elasticity and increase its fragility. As demonstrated by Song et al. (2023) study, a hydrogel with diminished mechanical strength is not viable for water purification systems due to its increased disintegration potential in an aqueous solution. Cellulose is highly eco-friendly and cost-effective compared to the light-absorbing material used in Guo and Yu's (2021) research. Contrastingly, for gold and silver production, significant amounts of energy and resources are used, and their disposal may result in metal accumulation in the environment. In addition, carbon blacks can also pose potential harm to the environment because they are derived from fossil fuels.

In summary, adding a light-absorbing material to optimized hydrogel matrices raises water temperature, hence increasing the efficiency of solar water purification. However, their use has environmental consequences like accumulation and can drive up the price of hydrogel production. Therefore, using cellulose from natural sources mitigates these drawbacks. Furthermore, the mechanical strength of the hydrogel is also reduced by these light-absorbing materials. However, when the hydrogel is synthesized from cellulose, the mechanical and structural properties are not tampered with, increasing the hydrogel's water absorption capacity.

#### *A comparative analysis of the synthesis and applications of hydrogels in solar water purification and environmental remediation*

One such study run by Song et al. (2023) focuses on hydrogel synthesis and its possible applications in environmental remediation and antimicrobials. This particular experiment examined the use of hydrogels for water purification by eliminating heavy metals, dyes, and organic contaminants from water. Song et al. (2023) used chitosan, alginate, and polyvinyl alcohol to synthesize their hydrogel; however, due to its abundance and eco-friendliness, my study employed cellulose as the basis material for hydrogel synthesis.

By combining chemical reagents like chitosan, alginate, and polyvinyl alcohol, Song et al. (2023) were able to develop hydrogels used in water filtering. The chemical and physical qualities of each of these materials determined whether or not they would be preferable in hydrogel synthesis. For instance, chitosan possesses various



advantages, including biocompatibility, biodegradability, and antibacterial characteristics that make it suitable for hydrogel synthesis. However, due to chitosan's chemical makeup, it is not suitable for an environmental application. This is due to chitosan being insoluble in water without the presence of an acid, making its incorporation into water filtering systems exceptionally challenging. However, A.L. Samman and Sanchez's (2021) research indicated that alginate hydrogels expand and degrade in water, and thereby their poor mechanical qualities would rule them out as a viable water purification option. The study also indicated that alginate degraded rapidly when subjected to mechanical stress from compounds like chitosan. Lastly, Song et al. (2023) employed the use of synthetic polymer polyvinyl alcohol to synthesize hydrogel, which is frequently utilized in hydrogel synthesis due to it being biocompatible, biodegradable, and very water-soluble. However, Wang et al. (2018)'s recent study differs from other studies. It indicates that polyvinyl alcohol has poor solubility and weak mechanical strength in water, which makes it unsuitable for use in water purification processes.

In contrast to Song et al. (2023), I opted for cellulose as the hydrogel synthesis foundation material, mainly due to the quantity and eco-friendliness of cellulose. According to a study by Patchan (2013), cellulose is the most prevalent biopolymer on earth. In addition, Patchan's (2013) study reveals that cellulose has high water solubility, is biocompatible, non-toxic, and possesses outstanding mechanical strength, making it a suitable material for water purification systems.

In summary, as presented above, materials used by Song et al (2023) study have their own unique chemical properties that make them suitable for water-purification. Additionally, cellulose-based hydrogels exhibit exemplary mechanical strength and solubility even in aqueous solution as presented in Zainal et al. (2021) study as compared to chitosan, which has both poor solubility and requires acid to increase solubility, and alginate, which has poor mechanical strength and disintegrate in aqueous solution.

## Materials and Methods

This experiment was designed to quantify the rate of water purification across a suite of differently colored hydrogels. Solar-powered water purification is a natural water purification technique in which fresh water is separated from contaminant molecules by evaporating the water molecules using solar heat (Altherr et al., 2008). The point of this type of method was to analyze and compare the performances of the differently colored hydrogels using their rate of evaporation, so observing the phenomena with direct laboratorial experimentation was the easiest way to analyze the data. Furthermore, the method applied allowed for direct observation of any possible changes to the appearance of the hydrogel and clarity of water produced (Zhou et al., 2019).

The hydrogel synthesis technique was also essential to the success of my experiment. The chosen approach had to guarantee that the hydrogels possess the proper swelling and mechanical properties. It required the aforementioned properties for my synthesized hydrogel to maintain its structural integrity and water-absorption capacity during solar desalination and water purification. The choice of watercolor dyes was also crucial, as it was based on the fact that none of the stains interfered with the hydrogel's properties, resulting in accurate and timely results.

## *Fabrication of cellulose-based hydrogels*

### *Materials*

The components utilized in synthesizing cellulose-based hydrogel were meticulously chosen based on their compatibility and capacity to form a stable hydrogel structure with desirable mechanical and swelling properties. Due to their biocompatibility, non-toxicity, and biodegradability, cellulose and epichlorohydrin (ECH) were used to synthesize cellulose-based hydrogel. I utilized NaOH as a crosslinker to form a three-dimensional network within my hydrogel. Therefore, the objective of the three-dimensional network was to give hydrogel mechanical strength and prevent its dissolution in water. Urea was utilized to optimize the swelling characteristics of my hydrogel, which will increase hydrogel capacity of retention.

Different natural and synthetic tinting techniques were employed to produce various hydrogel colors. I decided to use watercolor dye, methylthioninium chloride, and graphite powder to create multiple shades due to their compatibility with hydrogel synthesis materials. Watercolor dye was also used due to its abundance in producing a vast array of colors. This enabled me to increase the light-absorption properties of the hydrogel, hence improving the efficiency of solar water purification. I purchased all of my reagents from Sigma Aldrich, a reputable online vendor of laboratory chemicals and reagents, to ensure accurate and reliable experimental results.

### *Fabrication process*

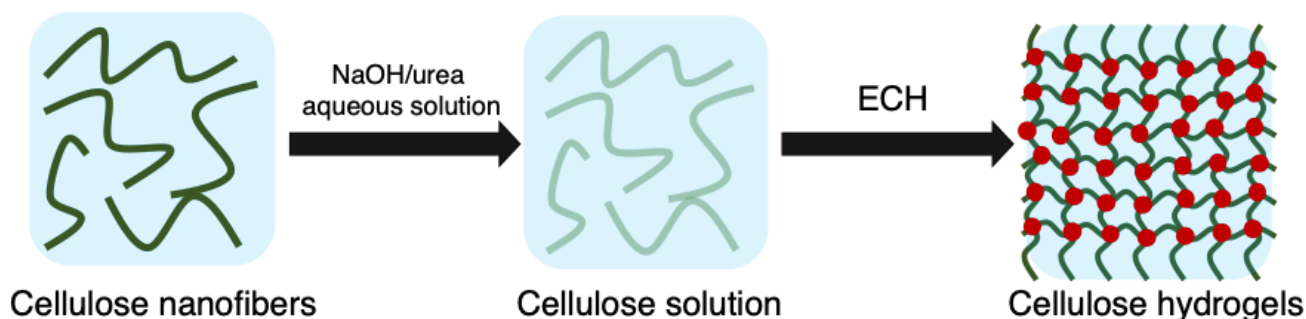
Firstly, to produce environmentally friendly hydrogel disks, I chose cellulose as the base material; because it comprises most plant tissue, cellulose is the most widely distributed organic compound and is extremely affordable (Aziz et al., 2022). Furthermore, cellulose hydrogels are biodegradable, thus giving them a clear environmental advantage over other polymers that may contain harmful synthetic chemicals or pollutants (Motloung et al., 2019).

To synthesize the hydrogels, the cellulose was first dissolved in water to create a base solution. Because cellulose is not easily soluble, 3.5 g NaOH and powdered urea were added to 40.5 g of water to help the cellulose dissolve. These two substances release large amounts of heat when dissolved in water and thereby accelerate the solubilization of cellulose. Once the cellulose was fully dissolved, ECH was added to solidify the hydrogel mixture from a liquid to solid. This process, known as gelation, produces chemical cross-links between the polymers in the structure. Subsequently, watercolor dyes and graphite powder were added after gelation to produce different tints in the gel matrix.

This hydrogel fabrication procedure was based on the procedure described by Zhang et al., who used NaOH and urea to fully solubilize the cellulose before adding ECH as a cross-linking agent. Zhang states that the ECH-induced gelation step is necessary because hydrogels are hydrophilic solids that must hold their shape during the water purification process. In that same vein, if the hydrogel was used as a non-gelatinized liquid, the solution would simply be diluted when water was added and the impure water would not be effectively evaporated.

Once ECH was added to the NaOH-urea-cellulose solution, the solution was evenly distributed between six labeled petri dishes and baked in an oven for four hours, as baking induces gelation and the consequent formation of hydrogel disks (Peidayesh





**Scheme 1:** A schematic diagram demonstrating the fabrication of differently-colored, cellulose-based hydrogels.

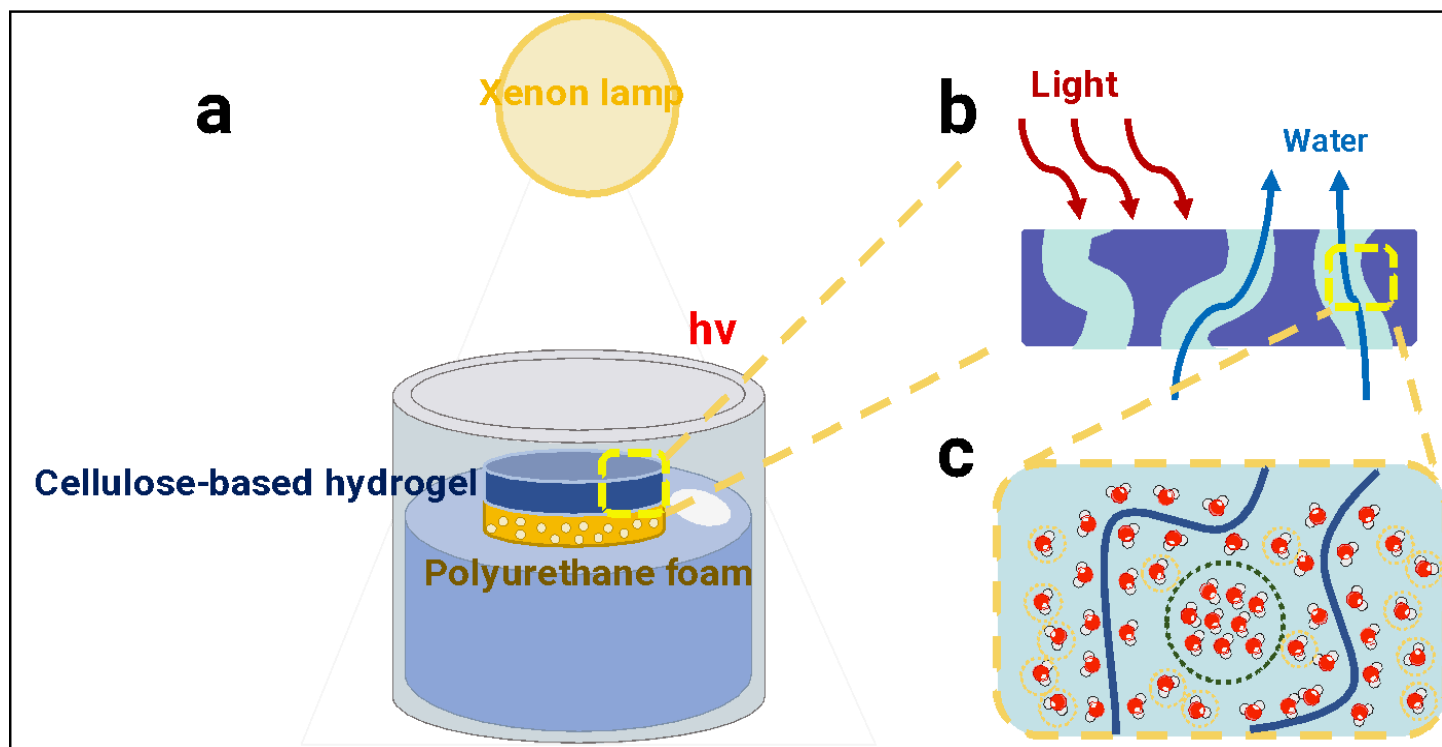
et al., 2020). However, because a strong base was used to dissolve the cellulose, the newly formed hydrogel disks were washed with distilled water three times over twelve hours. This would prevent excess hints of leftover dye or particulate matter, which may have been picked up during the baking stage.

*Measuring the effect of hydrogel color on evaporation rate*

To determine the efficacy of water evaporation, a xenon lamp was utilized. Unlike natural sunshine, which varies in intensity and duration, the xenon lamp gave me greater control over experimental settings without extraneous mistakes. Secondly, according to Sharshir et al. (2020)'s study on the effect of temperature and humidity on hydrogel-based solar water evaporation, all hydrogel systems should maintain an environment of 15 °C and 30% relative

humidity during the experiment. This is owing to the sensitivity of hydrogel to variations in temperature and humidity, which might lead to incorrect experiment results. The work done by Pan et al. (2022) also supports using xenon lamps because they generate sun-like conditions that make them dependable and accurate light sources for replicating solar water purification systems.

Due to research time limits and the need for accurate data, the evaporation rate was recorded every six hours as it would not be expected to shift drastically over short periods. As illustrated in Scheme 2a, this measurement frequency was employed to determine an accurate measurement of the water evaporation rate in each hydrogel disk. A beaker containing 150 mL of water and hydrogel was placed beneath the xenon light. The water evaporation rate was then determined by dividing the water lost from the beaker by the period that passed.



**Scheme 2:** Schematic diagram showing (a) the experimental system, with a hydrogel resting on polyurethane foam in a beaker underneath a xenon lamp; (b) the enlarged hydrogel, and (c) the enlarged pores of a hydrogel.

### *Water evaporation efficiency across differently colored hydrogels*

I suspended cellulose-based hydrogels on a layer of polyurethane foam to ensure direct exposure to the xenon lamp, since they tend to settle in water. I monitored evaporation rates every six hours for 24 hours to better understand hydrogel's efficacy in water purification. This was achieved by taking measurements at the intervals specified in scheme 2a. As a comparison standard, it was decided to include a beaker devoid of the hydrogel. This would allow me to calculate how much faster the hydrogels evaporated water than the baseline.

## Results and Discussion

### *Water evaporation efficiency across differently colored hydrogels*

The control beaker exhibited a low evaporation rate, with only 8 mL of water evaporating in the first 6 hours (Figures 2a, 3). In the three subsequent 6 h periods, evaporative water loss was 8 mL, 12.5 mL, and 11.5 mL, respectively. Thus, the evaporation rate remained constant for the first 12 hours but then increased by 56% for the subsequent 6 hours, presumably because the water temperature was increasing with longer-term exposure to the xenon lamp. The evaporation rate then decreased slightly for the final six hours, potentially because continuous evaporation had increased the saturation vapor pressure.

The addition of hydrogels to the beaker was expected to increase the evaporation rate relative to the control. As described in the literature review, hydrogels can absorb solar energy and convert it into heat, thereby increasing the temperature of the surrounding water and thus the rate of evaporation. Moreover, due to the porous structure of hydrogels, water molecules can move freely from the interior of the hydrogel to its surface, where they can evaporate into the surrounding environment. This porous structure also facilitates the diffusion of water vapor out of the hydrogel, further increasing the rate of evaporation. Additionally, evaporation enthalpy intermediate water in hydrogels is much lower than free water (Scheme 2).

The experiment findings were in line with the literature review. Transparent hydrogel placed on polyurethane foam in the beaker resulted in faster water evaporation than the control beaker. 20 mL of water evaporated during each of the first two 6-hour measurement periods. Like the control beaker, the initial evaporation rate remained constant for the first 12 hours. However, the rate was noticeably faster than the control. Only 10 mL evaporated in the subsequent 6 hours, and only 13 mL in the final measurement period. Put differently, the evaporation rate decreased by almost 75% after the first 12 hours. These findings significantly differed from the control. Evident from the study, in the control's case, evaporation rate surged after the first 12 hours. Indeed, the low evaporation rates over the second 12-hour period were quantitatively similar to the evaporation rates from the control. The decrease in evaporation rate was likely stemming from the experimental flaw where the hydrogel suspended on the polyurethane foam was no longer in contact with the water after the first 40 mL had evaporated. Nevertheless, despite this flaw, the rapid evaporation rate observed during the first 12 hours of the study matched the results foretold in the presented extant literature.

I then tested the effects of hydrogel color on the evaporation rate. Figures 2c and 3 show the amount of water evaporated from a beaker with a red hydrogel suspended on

polyurethane foam. During the first six hours, 25 mL of water evaporated, which is 25% more than with the transparent hydrogel. During the subsequent six hours, 38 mL of water evaporated, which is twice the amount that evaporated from the transparent hydrogel and nearly five times more than the control. An additional 37 mL evaporated over the third six-hour period, and evaporative loss was 41 mL over the final six-hour period. Because this substantially higher evaporation rate was achieved with the simple addition of a red dye, red hydrogels may be a valuable tool in solar water purification systems; indeed, the cost of the dye is considerably lower than the initial cost of making hydrogels or using other energy sources for solar water evaporation. Overall, the red hydrogel was 2-3 times more efficient than the transparent, non-dyed hydrogel, with only a fractional added cost.

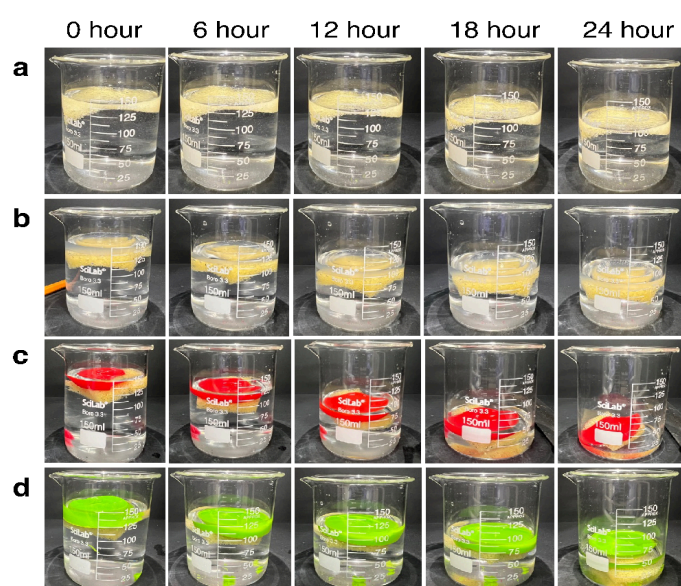
Figure 2d shows evaporation over time with a green hydrogel. Only 20 mL of water evaporated over the first six hours with the green hydrogel, which represents no improvement over the transparent hydrogel. However, evaporative loss increased to 28 mL during the second six hours, suggesting that the green dye may nevertheless increase the evaporation rate. Evaporative water loss decreased to 12 mL during the subsequent six hours and then continued to decrease to the end of the experiment, similar to the results observed with the transparent hydrogel.

As expected, the colored hydrogels in this study were more effective at promoting evaporation than the transparent hydrogel. When a colored hydrogel is exposed to a xenon lamp, it absorbs light at wavelengths that correspond to its absorption spectrum. The absorbed light is converted into heat energy, which increases the temperature of the hydrogel and, correspondingly, the surrounding water, and the warmer water then evaporates more quickly. Colored hydrogels may also have a higher surface area than transparent hydrogels due to their porous structure, which can further increase the rate of water evaporation. Conversely, transparent hydrogels are not as efficient at absorbing light and converting it into heat because they lack light-absorbing pigmentation. As a result, the temperature of a transparent hydrogel may not increase as much as with a colored hydrogel, resulting in a lower evaporation rate.

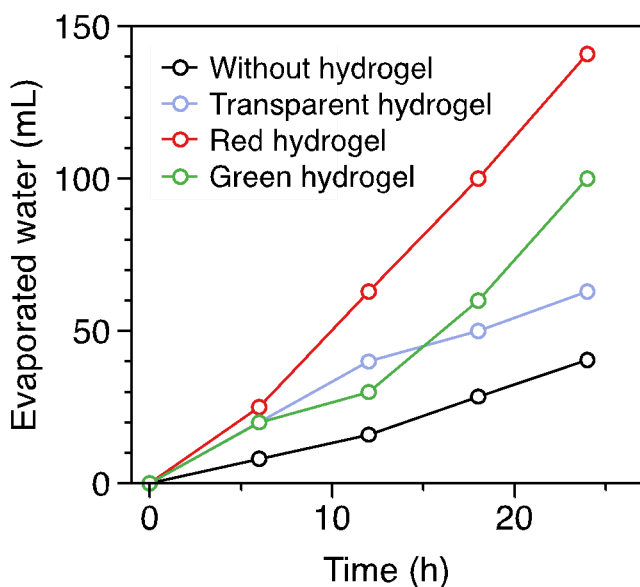
The choice of color also had a significant impact on the hydrogel's ability to absorb and convert light into heat. In this study, I found that red hydrogels exhibited a higher water evaporation rate than green hydrogels when incubated under a xenon lamp. Different dyes have different absorption spectra, meaning they absorb different wavelengths of light. Because red light has a longer wavelength than green light, it can penetrate deeper into the hydrogel and be converted into heat more efficiently, resulting in a higher temperature increase and therefore increased evaporation rate. Conversely, green light has a shorter wavelength and is absorbed less efficiently by the hydrogel, green hydrogels do not cause as pronounced of a temperature increase and are therefore associated with a slower evaporation rate. The choice of color becomes particularly important in applications such as solar-powered water evaporation, where a color that efficiently absorbs and converts light into heat can help optimize the evaporation process and improve its efficiency.

In addition to the effects of color alone, the specific dye molecules used to create the color may also affect the amount of light energy a hydrogel can absorb. The red dye used in the red hydrogel may have a higher absorption coefficient in the red region of the

spectrum than the green dye does in the green region of the spectrum, resulting in more efficient light absorption and heat conversion.



**Figure 1:** A series of photos capturing the evaporation of water in a beaker under a xenon lamp over time, taken every 6 hours: (a) Only polyurethane foam, (b) transparent hydrogel, (c) red hydrogel, and (d) green hydrogel.



**Figure 2:** A graph depicting the rate of evaporated water in mL per hour-designated periods.

## Conclusion

As the threat of climate change looms closer, the need for innovative, efficient, and sustainable water purification systems has become increasingly urgent. Therefore, in this study, I have demonstrated that the color of hydrogels can have a significant impact on their ability to absorb and transfer light energy, which, in turn, affects their evaporation rate in a water purification system. To be more specific, red hydrogels proved to be the most effective at maximizing

evaporation while keeping additional costs of materials to a minimum. By shedding light on the role of hydrogel color in the purification process, this research may help to build awareness about the importance of preserving scarce water resources. The results of this study provide valuable insights into the vast potential of hydrogels as a sustainable and cost-effective solution for water purification in the face of the growing threat of pollution and climate change.

## Appendix of Vocabulary

For the purpose of clarification, crucial terms used in this study have been defined. The following terms are:

**Carbohydrate.** A group of organic compounds that include sugars, starches, and cellulose, all of which consist of carbon, hydrogen, and oxygen atoms and serve as a major source of energy in living organisms.

**Cross-linked.** The condition of having chemical bonds between two or more polymer chains that are typically formed through a series of chemical reactions or physical interactions, which can modify the physical and mechanical properties of a material.

**Gelation.** The process of forming a gel that involves the formation of a three-dimensional network of cross-linked molecules that can trap and retain liquid (often water) within its structure.

**Hydrogel.** A type of cross-linked polymer composed of a network of hydrophilic polymer chains capable of retaining large amounts of water, often with physical properties such as high elasticity, softness, and porosity.

**Hydrophilic.** Having a strong affinity for water molecules and being able to attract and hold them within a structure for long periods of time without disturbance.

**Porous.** To have small spaces or pores within a structure that allow liquids or gases to pass through, often used in reference in materials with a high degree of permeability.

**Polysaccharide.** A large molecule consisting of multiple sugar units linked together, which are commonly found in carbohydrates and serve various functions in biological processes.

**Purification.** The act of removing impurities, contaminants, or pollutants from a substance to obtain a cleaner, more refined final product.

**Soluble.** Being capable of dissolving within a particular solvent to form a homogeneous mixture.

**Spectra.** A range of different frequencies or wavelengths of electromagnetic radiation that are used to describe patterns of light and/or color produced by a specific light source.

**Wavelength.** The distance between two adjacent points or troughs of a wave, usually in terms of electromagnetic radiation properties, such as light.

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# HeLa Cells' Interaction with Nanoparticles Zinc Oxide and Titanium Dioxide

COURTNEY O'KANE

JAMES MARTI

MATTHEW JOHNSON

KRISTYN VANDERWAAL MILLS

*SAINT PAUL COLLEGE*

*UNIVERSITY OF MINNESOTA NANO CENTER*

Interest in nanoparticles has grown significantly as concerns of their potential toxicity to human cells and aquatic life have been raised. Harmful impacts of engineered nanoparticles are of great interest as they are used in many common products from pharmaceuticals to sunscreen. Nanoparticles are materials with a diameter less than 100 nanometers, which makes their penetration of cell membranes quick and efficient. This work explored the impacts of zinc oxide (ZnO) and titanium dioxide (TiO<sub>2</sub>) nanoparticles on living HeLa cells. The goal of the experiment was to determine whether cell mortality would increase with increasing nanoparticle concentration, and if a crucial nanoparticle concentration exists that results in increased cell mortality. Cells were prepared, cultured, subcultured, and analyzed observing strict aseptic technique. After one month of successful HeLa cell replication, ZnO and TiO<sub>2</sub> nanoparticle dispersions were prepared at a range of dilutions and introduced to the HeLa cells. The nanoparticles' effects on cells were established using hemocytometry to determine cell viability and cell mortality. The results suggest that cell mortality increased monotonically with higher concentrations of TiO<sub>2</sub>. In contrast, lower concentrations of ZnO increased cell viability, while increased cell mortality was observed at the higher concentrations of ZnO. Given that results with ZnO were less conclusive, more research is needed to address the impact of ZnO on HeLa cells. Future studies will aid in understanding the implications of nanoparticles on living cells.

**Keywords:** Nanoparticles, HeLa Cells, HeLa Cells and Nanoparticles, Titanium Dioxide and HeLa Cells, Zinc Oxide and HeLa Cells, Health Implications and Nanoparticles, Nanoparticle Effects, Environmental Impacts and Nanoparticles, Toxicity of Engineered Nanoparticles

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## Introduction

Interest in nanoparticles and their effect on aquatic life and human cells has led to increased research on the environmental toxicity of nanoparticles [1]. Nanoparticles are defined as particulate materials with a diameter between one and 100 nanometers and may contain a wide variety of compounds, including metals, inorganic oxides, and organic materials. While some nanoparticles occur naturally, most concern is centered on the environmental health impacts of engineered nanoparticles that are commonly used in a wide range of products, from industrial coatings to cosmetics to pharmaceuticals [2]. Two widely used engineered nanoparticles are titanium dioxide (titania, TiO<sub>2</sub>) and zinc oxide (ZnO); these materials are frequently used in toothpaste, sunscreen, and various types of paints and plastics [3]. Nanoparticles can reflect and disperse UV light making them ideal for use in sun protection products [3]. Nanoparticle

titania has further uses in orthopedic implants, oncological medicines, the manufacturing of electronics, and water decontamination. According to Brandão et al. [4], the International Agency for Research on Cancer (IARC) classifies TiO<sub>2</sub> as a Group 2B carcinogen, considered "possibly carcinogenic to humans." TiO<sub>2</sub> specifically has been seen to increase free radical production and tissue inflammation [4].

Past research involving nanoparticles has centered on using phagocytes, macrophages, monocytes, and dendritic cells as models for understanding nanoparticle effects on human cells [5]. According to Brandão et al. [4], the following four human cell lines are good models for genotoxicity research: neurons, lung cells, glial cells, and liver cells. In previous studies, human cell lines of the respiratory system were the focus of experimentation, as concern about nanoparticle toxicity originally focused on risks due to

inhalation [4]. In more recent studies, scientists have observed how nanoparticles enter human cells after being ingested orally or applied topically [6]. Human cell lines derived from lung, brain, kidney, spleen, and liver tissue are becoming central to nanoparticle genotoxicity research as understanding of nanoparticle toxicity increases [4].

Liposomes are another important and influential human cell model used to investigate the health impacts of nanoparticles. According to Wang and Wang [7], the liposome was the first platform used to study human cell interaction with nanoparticles. Liposomes are widely used in research because therapeutic drugs can easily enter their outer membranes [7]. Copper nanoparticles in conjunction with serum albumin have been widely successful for use in chemotherapy treatments used to target aggressive breast cancer [8]. Copper nanoparticles are ideal for use in oncological medicines because of their ability to enter and disrupt cancer cells with negligible harm to healthy cells [8]. Nanoparticles are also being used to detect pathogens (surface antigens on invaders are detected through the magnetic properties of nanoparticles) and for passive targeting in cancer treatments, a technique where tumors are identified through nanoparticle collection in cancerous tissue [7].

The small scale of nanoparticles makes their penetration of cell membranes fast and efficient, making them preferred for use in nanomedicine and biomedical treatments. For example, nanoparticles are widely used to treat aggressive glioblastoma brain cancer cells [3], [9]. Nanoparticles are used to treat transmissible disease and illnesses associated with the cardiovascular and respiratory systems [5]. According to McMillan et al. [5], nanoparticles have been successfully used as medicinal implants and in the restoration of damaged tissue and joints. They are also increasingly useful for identifying issues within the central nervous system.

Of particular interest in studying nanoparticle-cell interaction are HeLa cells. HeLa cells have been used in many experiments involving nanoparticles as they provide an excellent human cell model for conducting research [10]. HeLa cells are an immortalized human cell line derived from cervical cancer cells that are widely used in biomedical research. HeLa cells are currently being used in research settings to create a vaccine for HIV and have contributed to many scientific advances in understanding leukemia, AIDS, and various types of cancer [11]. HeLa cells proved instrumental in developing a vaccination for polio [12] and in better understanding immune system activity after vaccination for COVID-19 [13].

This work investigated the effect of titania and zinc oxide nanoparticle dispersions on HeLa cell cultures in vitro. The experiment examined the cell toxicity of different concentrations of  $\text{TiO}_2$  and  $\text{ZnO}$ . A goal of the experiment was to determine whether cell death would increase with increasing nanoparticle concentration, and whether there was a critical nanoparticle concentration of either  $\text{TiO}_2$  or  $\text{ZnO}$  which indicated the onset of cell mortality.

## Experiment

Research was performed in the biosafety level 2 labs of the University of Minnesota Nano Center from January 2022 to May 2022. Biosafety level 2 (BSL-2) containment was required for

protection against human disease agents and human papilloma virus associated with HeLa cells.

## Materials and Equipment

The He-La cell line employed in this study was obtained from the American Type Culture Collection (ATCC). Cells were kept frozen at  $-196^\circ\text{C}$  for ongoing use in the Nano Center lab until harvested and cultured. The HeLa cells used in the study were derived from this in-house frozen stock and were thawed using a warm water bath. Cells were then cultured in a  $\text{CO}_2$  incubator (Thermo Scientific) using T75 culture flasks. Cell subcultures were transferred to 6-well plates with 2 mL wells for nanoparticle exposure and cell imaging. Separation of cell suspensions was done using a Beckman XR-30 refrigerated centrifuge. All HeLa cell handling was carried out in a BSL-2 biosafety cabinet. Nanoparticle dispersions of zinc oxide ( $\text{ZnO}$ ) and titania ( $\text{TiO}_2$ ) were obtained from Sigma Aldrich (St. Louis, MO) and were diluted to achieve physiological pH and a low nanoparticle concentration, as described below. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS), obtained from Fisher Scientific, were the chosen growth media. Washing and dilution was carried out using 1 X phosphate buffered saline (PBS). Trypsin was used to separate cells adhered to the culture flasks during subculturing. Trypan blue was the staining agent used for hemocytometry and cell counting.

For nanoparticle dilution preparation and administration, a pH probe and six 50 mL sealed test tubes for dilution storage were used. A Leica DMI series inverted microscope with Leica imaging software were utilized for cell analysis, cell observation, cell photography, and cell counting. Hemocytometry slides, coverslips, and worksheets to perform desired calculations were used for cell counting.

## Methods

### *Cell Thawing and Cell Preparation*

Complete growth media was prepared using DMEM + 10% FBS and warmed to  $37^\circ\text{C}$  in a water bath. Two vials of HeLa cells were removed from the liquid nitrogen storage and thawed to  $37^\circ\text{C}$  observing proper sterilization and aseptic handling techniques. The thawed cell suspensions were transferred into 15 mL sterile centrifuge tubes, into which 10 mL of complete growth media was slowly introduced. The cell suspension was spun for three minutes at 500 rpm and the supernatant was removed in the biosafety cabinet. The concentrated cell contents were placed in two separate T75 flasks, and 15 mL of complete growth media was added to each flask. The flasks were incubated at  $37^\circ\text{C}$  with 5%  $\text{CO}_2$ . Confluence checks were performed every 48-72 hours. The HeLa cell cultures grew for six days before confluence was high enough (75%+) for the first media change.

### *Well Plate Preparation*

A solution of DMEM + 10% FBS, PBS, and trypsin was warmed to  $37^\circ\text{C}$ . The DMEM/FBS solution was removed from each T75 flask with a 10 mL pipette and discarded. The cells were then washed with 3 mL of PBS to remove the FBS, which acts as a trypsin inhibitor. Three mL of trypsin were added to each flask and incubated for 10 minutes at  $37^\circ\text{C}$  to detach the HeLa cells from the surface of each flask. After incubation, 7 mL of complete growth media was added

to the flask. A micropipette was used to remove a small volume of the suspended cells from the T75 flask. The pipetted cells from the T75 flask were then placed into each well of a fresh 6-well plate, along with 2 mL of complete growth media. The well plates were rocked gently back and forth to mix components and were incubated at 37°C for 48-72 hours.

#### Cell Culture Maintenance

Cell cultures in the T75 flasks and 6-well plates were checked biweekly to monitor cell viability and to calculate confluence. Cells were also carefully monitored to ensure that no contamination had occurred. Each time confluence reached approximately 75%, a media change was performed, as follows. A solution of DMEM/FBS and PBS was brought to 37°C. The spent growth media from each T75 flask or 6-well plate was removed and added to the biowaste container housed in the biosafety cabinet. The remaining cells were washed one to two times with 0.5 mL PBS (for the 6-well plates) or 3 mL PBS (for the T75 flasks). The PBS rinse was discarded and growth media was added to each 6-well plate (2 mL) and each T75 flask (15 mL). The refreshed cultures in the plates or flasks were incubated at 37°C until confluent, after which they were subcultured to allow for controlled growth.

#### ZnO and TiO<sub>2</sub> Dispersion Preparation

The initial pH values of the ZnO and TiO<sub>2</sub> dispersions were measured using a Mettler pH probe. As received, the ZnO and TiO<sub>2</sub> dispersions were pH 8.318 and pH 3.428, respectively. Due to the fact that these pH values were outside the physiological pH of 7.0, it was determined adverse effects on HeLa cells could occur. To mitigate the influence of unwanted variables, the dispersions were diluted to a pH of 7.0 to limit unwanted cell damage. Dilutions were kept in sealed 50 mL test tubes and refrigerated for use throughout the study. The 50 mL test tubes were rocked back and forth before each application of use to ensure even distribution of nanoparticles when introduced to HeLa cells. The diluted dispersions of ZnO and TiO<sub>2</sub> were prepared at three different concentrations. (See Table 1.)

Nanoparticle	Nanoparticle Mass	Media Volume
<b>ZnO (x 1)</b>	0.5 µg	26.999 mL DMEM + 10% FBS
<b>ZnO (x 3)</b>	1.5 µg	26.991 mL DMEM + 10% FBS
<b>ZnO (x 5)</b>	2.5 µg	26.998 mL DMEM + 10% FBS
<b>TiO<sub>2</sub> (x 1)</b>	0.5 µg	26.995 mL DMEM + 10% FBS
<b>TiO<sub>2</sub> (x 3)</b>	1.5 µg	26.999 mL DMEM + 10% FBS
<b>TiO<sub>2</sub> (x 5)</b>	2.5 µg	26.997 mL DMEM + 10% FBS

**Table 1:** Nanoparticle ZnO and TiO<sub>2</sub> dispersion preparation with nanoparticle mass (µg) in relation to media volume of DMEM/FBS.

The HeLa cell cultures in the 6-well plates were checked for confluence before adding the diluted nanoparticle dispersions. The existing growth media in the 6-well plates was removed and the cells were washed one to two times with 0.5 mL PBS. Two mL of each dispersion listed in Table 1 was added to each well in each 6-well

plate. The cell-nanoparticle mixtures were then incubated at 37°C for four days to allow for growth.

#### Cell Characterization

The cell cultures treated with nanoparticle dispersions were imaged every four days using a Leica DMI inverted microscope with Leica Application Suite (LAS) software. The images were used as a visual indicator of cell viability and cell mortality.

Cell culture viability and mortality were measured using hemocytometry. To analyze cultures from the T75 flasks and 6-well plates, the old growth media was removed and the cells were washed with PBS. Trypsin was added to the cultures in each flask or well and the trypsinized cells were incubated for 10 minutes at 37°C to detach the cells from the flask or well surfaces. New complete growth media was then pipetted into each flask or well. The complete growth media and trypsinized cells were transferred to a microcentrifuge tube along with 100 µL of the Trypan blue staining agent and gently agitated. A small volume (15-20 µL) of this mixture was transferred from the microcentrifuge tube to a hemocytometry slide. A coverslip was placed over the hemocytometry slide to seal the sample, and the slide was placed on the stage of the inverted microscope. Cell counting was performed manually to quantify dead and viable cells.

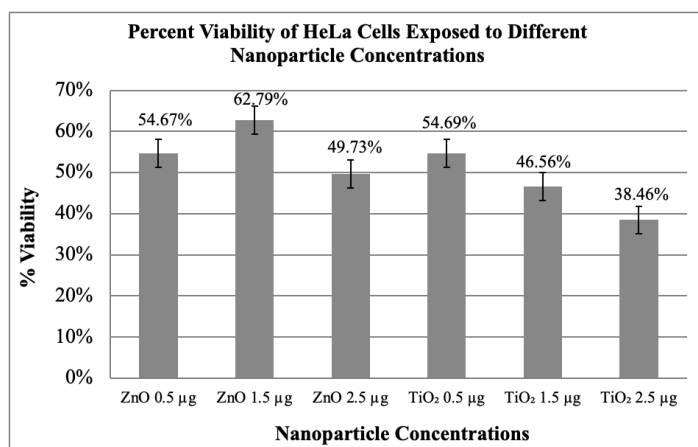
Based on hemocytometer images collected with the microscope, the following metrics were obtained: number of viable cells, number of dead cells, percent viability, number of cells per square, and cell concentration (cells/mL). Calculations dictated the amount of growth media and trypsinized cells (in µL) to be added to each well of a 6-well plate after trypsinization in preparation for exposure to nanoparticles. The calculation sought to determine  $V_1$  (the amount of trypsinized cells to be added to each well for exposure to nanoparticles) using the following calculation:  $V_1 = C_2V_2/C_1$ . The number of viable cells (the number of living cells counted through hemocytometry) was assigned the initial concentration ( $C_1$ ). The final concentration was held constant across trials at 200 cell/mL ( $C_2$ ). The final volume of growth media and trypsinized cells was also held constant at 2000 µL ( $V_2$ ). With these three known variables, the volume of growth media + trypsinized cells to be added to each well ( $V_1$ ) was established. After  $V_1$  was determined and the proper amount of cell suspension added to each well, 2 mL of fresh growth media were then pipetted into each well and incubated at 37°C for 48 hours. Subculturing of the T75 flask was then performed through removing 9.5 mL of the growth media and trypsinized cells. A fresh 14.5 mL of new growth media was then added to the T75 flask and the flask was incubated at 37°C for 48 hours.

Other calculations were also performed following hemocytometry to determine percent viability. Assessment of total viable cells and total non-viable cells dictated the percent viability through a simple calculation: total viable cells/total cells x 100%. (The total cells equaled the number of viable and non-viable cells combined.) The average number of cells per square of each hemocytometer slide, the dilution factor, and the total concentration of cells per T75 flask or 2 mL well were also found using the previously cited calculations to reach conclusions.



## Results

HeLa cells were exposed to ZnO and TiO<sub>2</sub> dispersions through identical trials over eight weeks. Hemocytometry and cell counting were conducted biweekly after HeLa cell exposure to nanoparticle dispersions. Preliminary results suggest that increasing concentrations of TiO<sub>2</sub> increased cell mortality monotonically. Percent viability of HeLa cells exposed to TiO<sub>2</sub> concentrations decreased from 54.69 % under 0.5 µg of TiO<sub>2</sub> to 46.56 % under 1.5 µg of TiO<sub>2</sub> and to 38.46 % under 2.5 µg of TiO<sub>2</sub>. Concentrations of ZnO at 1.5 µg *increased* cell viability (62.79 % viability) while the highest concentration of ZnO at 2.5 µg increased cell mortality (49.73 % viability). Concentrations of 0.5 µg of ZnO had little effect on HeLa cells with a percent viability of 54.67 %, mirroring the percent viability of HeLa cells exposed to 0.5 µg of TiO<sub>2</sub>. (See Table 2 for a graphical representation of results.)



**Table 2:** HeLa cell viability under 0.5 µg ZnO, 1.5 µg ZnO, 2.5 µg ZnO, 0.5 µg TiO<sub>2</sub>, 1.5 µg TiO<sub>2</sub>, and 2.5 µg TiO<sub>2</sub>.

On week five of the study, a large cluster of cells were observed, indicating cell splitting of the HeLa cells or a possible increase in viability for the cells exposed to the ZnO and TiO<sub>2</sub> nanoparticles. Large masses of HeLa cells were observed under the microscope again at week six, indicating further cell aggregation. Outside of cell splitting or increases in viability, it is possible that the HeLa cells were disturbed during transfer to the incubator, as abrupt agitation can cause cell clumping. No definitive conclusion was reached concerning the aggregated cells. At the end of the study, a positive control treatment tested the impact of actinomycin D on HeLa cells. As expected, actinomycin D caused complete cell death of the HeLa cells.

## Discussion

The study sought to quantify the effects of ZnO and TiO<sub>2</sub> nanoparticles on HeLa cells. The objective of the experiment was to determine whether cell death would increase with increasing nanoparticle concentration, and whether there was a critical nanoparticle concentration of either TiO<sub>2</sub> or ZnO which indicated the onset of cell mortality. The experiment showed that exposure of HeLa cells to TiO<sub>2</sub> had a significant toxic impact on the cells in the study, while applications of ZnO affected HeLa cells to a lesser degree. Cell mortality increased monotonically in the presence of

increasing TiO<sub>2</sub> concentrations, but no such trend was observed for cell exposure to ZnO. The latter finding suggests the need to explore a wider range of ZnO concentrations through research, with the goal of establishing the relationship (or lack thereof) between ZnO nanoparticle exposure and HeLa cell mortality. Specifically, more studies are needed to understand the counterintuitive increase in cell viability of HeLa cells exposed to ZnO concentrations at approximately 1.5 µg.

The observed increase in HeLa cell mortality at higher levels of TiO<sub>2</sub> suggests the need to investigate whether TiO<sub>2</sub> nanoparticles may have carcinogenic effects on human cells or human tissue. Likewise, observed increases in HeLa cell mortality at the highest levels of ZnO nanoparticle exposure also indicates that future research needs to determine whether there might be a threshold value of zinc oxide nanoparticle exposure above which cell mortality accelerates.

This study highlighted only two of the many nanoparticle formulations that are used in industry and consumer products, and to which workers, consumers, and the environment are being exposed to regularly. Zinc oxide and titanium dioxide were deemed a practical choice in beginning research on nanoparticle toxicity due to the fact that concerns of the impact of ZnO and TiO<sub>2</sub> nanoparticle exposure are already established and growing in the scientific and medical fields. However, research is required to identify the implications of biological exposure to all types and compositions of nanoparticles. Future studies that utilize zinc oxide, titanium dioxide, and other common nanoparticles will aid to understand the comprehensive consequences of nanoparticles on human cells, human tissue, and ultimately, the environment.

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I dedicate my work in this study to Henrietta Lacks. Mrs. Lacks died not knowing how greatly she would impact science; it is with my sincere gratitude that I was given permission to use HeLa cells.\* I want to express a multitude of thanks to Dr. James Marti for his co-authorship, advising, and generosity in inviting me to use the Bio-Nano Lab, and to Matthew Johnson, who offered excellent guidance, mentorship, and training. Lastly, it is because of Dr. Kristyn VanderWaal Mills that I was introduced to this amazing opportunity – her support throughout this study made my work possible.

\*The isolation and immortalization of HeLa cells changed the scientific landscape in the 20<sup>th</sup> century. HeLa cells, named for Henrietta Lacks, were first collected by two Johns Hopkins researchers in February, 1951, and through the efforts of later researchers, were established as a standard for cell laboratory propagation and studies [14]. The original HeLa cells were obtained from 31-year-old patient Henrietta Lacks, who was being treated for cervical cancer at Johns Hopkins Hospital [10]. The cells from Henrietta Lacks were obtained without her knowledge, as permission was not required for cell harvesting in 1951. Henrietta Lacks later died from the cancer on October 4, 1951.

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# Embracing Genetically Modified Crops for Global Prosperity

**YOUSIF DHIA**

*PENNSYLVANIA STATE UNIVERSITY*

This paper advocates for the global implementation of genetically modified crops, highlighting their substantial benefits and addressing prevalent controversies. The primary research question explores their impact on agricultural productivity, nutritional enhancement, and pesticide reduction. Utilizing a comprehensive literature review, the study reveals significant increases in crop yields and productivity, particularly in developing countries. It also emphasizes the nutritional benefits of biofortification, exemplified by  $\beta$ -carotene-enriched rice to combat vitamin A deficiency. Furthermore, the paper discusses their environmental advantages, such as reduced pesticide use and associated health risks. Addressing safety concerns, the research indicates a broad scientific consensus on the safety of GM foods, supported by major health organizations. Despite ecological concerns about biodiversity, studies show minimal adverse effects. The paper concludes by highlighting future prospects in genetic engineering, including cisgenesis and genome editing, which promise to further revolutionize agriculture. These findings suggest that genetically modified crops are a crucial tool for achieving global agricultural prosperity and call for policy reforms and public education to support their adoption.

**Keywords:** Agricultural Biotechnology, Agricultural Sustainability, Biofortification, Crop Improvement, Genetic Engineering, Genetically Modified Crops, Global Agriculture, Nutritional Enhancement, Pesticide Reduction

## Introduction

Amidst humanity's enduring voyage into the realms of scientific and technological progress, possibly one of our greatest achievements is the ability to change the fundamental hereditary code of living organisms, serving a pivotal role in crop improvement within global agriculture [1]. This discovery has been applied to various organisms, but one of the most prominent and practical applications is the genetic modification of the food we eat—specifically, the use of genetic modification to enhance plantation growth and resistance. Tampering with the DNA of our food has sparked much controversy, suspecting the unknown and risky consequences of such an action. Many countries that would heavily benefit from genetically modified crops have still not implemented them within their agriculture [2]. In this paper, I argue for genetically modified crop implementation by showing their contributions to increasing production, enhancing nutritional value, reducing pesticide usage, and addressing controversies surrounding safety, biodiversity impact, and pest resistance, as well as exploring the promising prospects of genetic engineering in the agricultural sector.

But before justifying such claims, we must know what genetic modification is and its history. Genetic modification is when the genome of an organism is modified using genetic engineering techniques to improve the existing traits or the introduction of a new trait that does not occur naturally in said organism [1]. Such discovery opened the window to take desired genes from plants that are not sexually compatible with other plantations for them to express desired genes.

## Genetic Modification's History

The first application of genetic engineering that was commercialized was in 1994 on tomatoes; only two years later, 1.66 million hectares of land were planted with genetically modified crops, and by 2020, that number became 185.6 million hectares [3]. This shows that genetically modified crops have had significant growth over time and are now a big contributor to many people's diets. This makes genetically modified crops the fastest crop technology to be adopted in modern agriculture [1]. One study estimates that 80 percent of processed food is genetically modified [4]; another shows that 90 percent of U.S. corn, upland cotton, and soybeans are genetically modified [5]. All of this shows how big an impact genetically modified crops have had on agriculture as a whole.

But how much has genetic modification benefitted crops in terms of production? A global meta-analysis of transgenic crops has shown that genetically modified crops have increased crop yields by 22 percent and increased farmer profits by 68 percent, which have been shown to be greater in developing countries than in developed countries [6]. This shows that not only have genetically modified crops greatly increased the productivity of global agricultural production, but have also significantly benefitted economic growth and prosperity, surprisingly and fortunately more so in developing countries. These benefits can only increase in correspondence to the status quo of countries that have not yet implemented genetically modified crops.

Getting into specifics, genetically modified crops have allowed the fortification of much-needed nutrients to various

common crops in populations that have deficiencies in certain vitamins and minerals. This process, known as biofortification, is one of the ways that genetically modified crops have the potential to greatly benefit large populations.

For instance, one of the first major advances in genetic modification was the implementation of  $\beta$ -carotene into rice.  $\beta$ -carotene is a leaf-specific pigment that the body can use to convert to vitamin A [7]. This was done as a countermeasure against vitamin A deficiency, a worldwide disease and the leading cause of preventable blindness in children; it has also been shown that it is correlated with a higher risk of severe disease and death [8]-[10].

Especially in impoverished populations that heavily consume rice, genetic modification can have substantial potential for preventing blindness. Unfortunately, even after almost 24 years of its creation [11], it has not yet been implemented in populations that would benefit the most from it, such as Southeast Asia [7]. I hope this will change in the future, as it has the potential to completely prevent vitamin A deficiency. This is only one of many nutritional fortifications that were made possible because of genetic modification; other examples include folate biofortification, iron biofortification, essential amino acids, and the elimination of trans fats from oilseed crops [1], [7]. Considering the short history of genetically modified crops, their nutritional value has endless potential for aiding these populations if they were implemented within them.

Besides nutritional fortification, the two main categories of genetically modified crop traits that are most used are tolerance to herbicides and resistance to insect pests [3]. It's important to point out what tolerance to herbicides means here: instead of the crop being altered to resist herbs and weeds (a very arduous task), the crops are instead resistant to the pesticides used upon them. While this might sound like it does not change much, it allows for much more efficient pesticide use and eliminates much of the damage done to crops through the excessive use of pesticides. For instance, herbicide-tolerant genetically modified maize (corn) has reduced active ingredient use by 220 million kg in the U.S. alone [3]. For cotton, it has reduced active ingredient use by 20.5 percent in Australia [3].

This is only one side of the coin. Looking at the other, and much more significant, side, genetic modification for insect resistance has revolutionized modern agriculture by reducing the reliance on chemical pesticides and, in turn, reducing the environmental footprint left by them. Specifically, it has lowered pesticide use on cotton by -338.9 million kg (29.9 percent) worldwide and has lowered the environmental impact of the pesticides used on cotton by 34.4 percent [3].

I can keep giving statistics on how much genetically modified crops have lowered the use of pesticides, but why is this significant? Pesticides have caused much harm to human health; they are known to cause extremely adverse effects, which can be acute as well as chronic. Some examples of acute health effects include diarrhea, rashes, blindness, dizziness, stinging eyes, nausea, blisters, and death; some examples of chronic health effects include reproductive harm, neurological and developmental toxicity, endocrine system disruption, birth defects, cancers, and immunotoxicity [12]. One of the primary goals of modern agriculture is to reduce or eliminate the use of pesticides due to these adverse effects, and genetic engineering has opened the possibility for

such an ambition. If these crops were to be implemented globally, humanity would be much closer to achieving such an ambition.

Moving on to the question that much of the controversies around genetically modified crops have asked, are they safe to consume? Before answering such a question, I have to first point out where most of the controversy surrounding genetically modified crops originated from, the "Seralini affair."

## The Seralini Affair and Genetically Modified Crop Safety

In 2012, a study was published on genetically modified crops that claimed adverse health hazards, such as (all symptoms found in rats, not humans) high tumor incidences, chronic kidney disease, increased liver congestion, and necrosis in males, and increased female mortality [13]. Since its publication, the study has received extreme criticism from the scientific community because of its flawed experimental design and faulty statistical analysis, eventually leading to the retraction of the article [1]. Two years later, the same group published nearly the exact same work as they did before [14], this time without retracting the article.

With very few exceptions, most studies showed no effects of transgenic food on animals like rodents, poultry, pigs, frogs, cows, and monkeys [15]-[17]. I would think that much of the controversy and concerns surrounding genetically modified crops originated from these two studies, but to answer the question stated previously, scientists generally agree that genetically modified foods are safe to consume, a view that is supported by the American Medical Association, the National Academy of Sciences, the American Association for the Advancement of Science, and the World Health Organization [7], [18]. This leaves no excuse for all countries around the world not to implement genetically modified crops for the betterment of global agriculture.

## Effects of Genetically Modified Crops on Biodiversity and the Environment

One of the most frequent concerns of genetically modified crops is their unknown and potentially adverse effect on biodiversity and the environment due to the inability to account for all possibilities of nontarget organisms when changing the genetic makeup of plantations. Specifically, many ecologists are debating their effects on nontarget invertebrates that are ecologically important to plant nutrient availability and recycling of organic matter [19]. Such organisms include earthworms, termites, woodlice, snails, millipedes, beetles, and mesofauna [20].

One primary subject of the debate is the *Bacillus thuringiensis* (*Bt*) transgenic crops, which have been genetically modified to include a gene from *B. thuringiensis*, a species of bacteria that lives in soil [21]. The gene that is inserted into the plant's genome allows the plant to express a Cry toxin that provides resistance against insect pests [19]. In a meta-analysis that considered a total of 6110 titles, it concluded that "there was no significant effect of *Bt* on soil invertebrates."

While this example may sound very specific and irrelevant, *Bt* crops are an extremely significant advancement in genetic modification and are one of the main genetically modified crops used commercially. For instance, 80 percent of all corn in the U.S. is genetically modified to include the *Bt* trait, and 85 percent of all cotton is *Bt* as well [22]. The fact that the *Bt* trait does not correlate



with any harm to biodiversity [19] leaves no reason for countries that haven't implemented or banned genetically modified crops.

One argument that can be made against pesticide resistance development through genetic modification is the causation of the development of resistance by the pests themselves due to the overreliance on the no longer harming pesticides. This is an argument that has solid ground, as much of the environmental gains associated with more efficient pesticide use have diminished over time, however, as of 2020, the adoption of herbicide-resistant genetically modified crops still shows a net environmental gain [3]. Considering the agricultural environment of countries that have not implemented them yet, there is still much potential for their environmental gain.

Not only this but many prospects in the field of genetic engineering could potentially revolutionize modern agriculture. Such prospects include Cisgenesis and intragenesis, and genome editing.

### Prospects of Agricultural Genetic Engineering

Cisgenesis and intragenesis allow genetic engineers to take only the desired genetic trait from the same crop species (in intragenesis some parts of the gene can be from different species), as conventional agricultural breeding does, only without the undesired traits that come along with it [1]. This practice has already shown great success, such as in blight resistance in potatoes [23] and scab resistance in apples [24]. Genome editing would allow genetic engineers to edit genes however they like, whether it be to replace, remove, or add a gene [1].

These tools open an infinite window of possibilities that have the potential to increase crop production, promote abiotic and biotic resistances in crops, meet consumers' nutritional needs, and eliminate every possible concern surrounding the genetic modification of plantations [1], leaving countries that have not implemented genetically modified crops yet with no argument. I hope to see global prosperity achieved through them, and to see no bans, restrictions, or concerns surrounding them in the future for the betterment of global agriculture.

### Conclusion

This paper has presented a comprehensive argument for the global implementation of genetically modified crops, demonstrating their substantial benefits in enhancing agricultural productivity, nutritional value, and environmental sustainability. Through a thorough literature review, we have established that they significantly increase crop yields and farmer profits, particularly in developing countries, and offer crucial nutritional benefits through biofortification, such as  $\beta$ -carotene-enriched rice to combat vitamin A deficiency. Additionally, they contribute to environmental sustainability by reducing the need for chemical pesticides, thus lowering associated health risks and environmental damage.

Addressing the controversies surrounding genetically modified crops, the evidence supports a broad scientific consensus on their safety, with endorsements from major health organizations like the American Medical Association and the World Health Organization. Concerns about biodiversity and ecological impact, while valid, have been shown to have minimal adverse effects when managed appropriately. Furthermore, the potential for future advancements in genetic engineering, such as cisgenesis and genome

editing, promises to overcome current limitations and enhance the benefits of genetically modified crops.

The findings of this paper advocate for policy reforms and public education to support the adoption of genetically modified crops worldwide. By leveraging the advantages of genetic modification, we can achieve global agricultural prosperity, improve food security, and address pressing nutritional deficiencies. The future of agriculture lies in embracing these technological advancements, ensuring that the benefits of genetically modified crops are realized on a global scale for the betterment of humanity and the environment.

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