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Inhibition of Quorum Sensing to Mitigate the Risks of Membrane Biofouling in Biological Wastewater Treatment

Priyanka Kakkar[1], Neeraj Wadhwa[2*]

Abstract

Membrane-based systems play a pivotal role in various applications, providing efficient separation, purification, and filtration processes across diverse industries such as water purification and desalination. However, this system faces major challenge of membrane biofouling impacting the efficiency and sustainability of such systems. Biofouling occurs when various microorganisms like bacteria, fungi, etc. adheres to the surface of membrane and form biofilm which results in reducing membrane permeability and increasing its operational cost. Various physical, chemical and biological strategies are known to prevent biofouling. Inhibition of quorum sensing is a recent trend based on the researches that shows quorum sensing as an integral part of biofilm formation. Bacteria's uses cell to cell communication mechanisms known as Quorum sensing, plays an important role in formation of virulence factors, biofilm. It is regulated by quorum sensing signalling molecules known as autoinducers like Autoinducing peptides, acyl homoserine lactones, Autoinducer-2. Various strategies can be adopted to inhibit quorum sensing: inhibition of Autoinducer synthase which is responsible for synthesizing autoinducers; inhibition/degradation of autoinducers and interfering with signal receptors. This article provides deeper knowledge of Quorum sensing (QS), methods of QS inhibition: Quorum quenching which involves the enzymatic degradation of QS signaling molecules, and the use of Quorum sensing inhibitor compounds in preventing membrane biofouling are discussed in detail. This article delves into the potential of disrupting quorum sensing pathways to prevent or reduce biofilm formation, thereby enhancing the performance and longevity of membranes in wastewater treatment. The mitigation of biofouling can revolutionize membrane-based systems, ensuring their sustainability and efficacy in water treatment processes.

Keywords: Membrane Biofouling, Quorum sensing, Quorum Sensing Inhibition, Waste water treatment

***Author for Correspondence**
Neeraj Wadhwa
E-mail: neeraj.wadhwa@mail.jiit.ac.in

[1]Research Scholar, Department of Biotechnology, Jaypee Institute of Information technology, A-10, Sector-62, Noida-, Uttar Pradesh, India.
[2]Professor, Department of Biotechnology, Jaypee Institute of Information technology, A-10, Sector-62, Noida-, Uttar Pradesh, India

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INTRODUCTION

Membrane-based wastewater treatment is an advanced and efficient technology that plays a crucial role in addressing the growing global water scarcity and environmental pollution challenges. This method involves the use of semi-permeable membranes to selectively filter and separate contaminants from wastewater, allowing clean water to pass through. Membrane based reactors have find application in reverse osmosis, desalination and microfiltration [1]. Membrane based reactors offers several advantages over conventional treatment methods such as, high effluent quality, smaller footprints, lesser sludge, automatic control, improved denitrification and nitrification process [2]. However, this system faces major challenge of membrane biofouling posing operational and economic hurdles in maintaining system efficiency [3].

Biofouling occurs when microorganisms, like bacteria, algae etc. attach to the membrane surface and form a biofilm. Biofilm is defined as the aggregation of microbial cells on the surfaces in a covering of extrapolymeric substance (EPS) [4]. Apart from microbial cells, biofilms also contain organic compound like carbohydrates, protein, amino acids, absorbed nutrients, metabolite products, lysis products, macromolecules such as lipids, polysaccharides, proteins, DNA, RNA. Additionally, anionic nature of the biofilms is attributed to uronic acids like mannuronic acids, and pyruvates linked with ketal [3–4]. Formation of biofilm is complex process with multiple steps: Reversible attachment; Irreversible attachment via flagella, pili, and fimbriae; Cell growth, Quorum sensing and EPS production; maturation of biofilm involving macrocolonies formation and finally dispersal of the cells to for new biofilm [5–7]. This biofilm can lead to decreased water permeability, increased trans membrane pressure (TMP), and reduced overall system performance [8].

Various strategies are employed to mitigate biofouling, including the use of antifouling coatings on membranes, periodic cleaning routines, application of chemical biocides (copper, silver, and tributyltin,), membrane relaxation, air scouring, standard backwash and surface modification. These strategies often face challenges as anti-fouling coating chemicals, biocides are toxic to the environment while surface modification reduces the toxicity but provides adherence microbes [2–3–9]. Recent researches focus on technologies having new control techniques, advanced sensors, Artificial intelligence based real time fouling detection. Furthermore, sustainable approaches have been gaining more attention to mitigate membrane biofouling with an increasing interest in Quorum sensing inhibition [10–11].

Bacteria’s utilize a mechanism known as Quorum sensing for their cell to cell communication. It plays an important role in the development and persistence of biofilms, secretion of extracellular polymeric substances (EPS), and therefore contributing to membrane biofouling [12]. Understanding the mechanisms of quorum sensing in membrane biofouling is essential for developing effective strategies to control and inhibit this phenomenon. This review paper aims to explore quorum sensing, its importance in membrane biofouling and methods of inhibiting quorum sensing as a strategy to control biofouling.

QUORUM SENSING

Quorum sensing (QS) is a phenomenon observed in bacterial species where they communicate with each other through the release and sensing of chemical signals. In quorum sensing, bacteria release signalling molecules called autoinducers into their environment. Various signaling molecules are found within bacteria, with three being predominantly observed: Acyl-Homoserine Lactones (AHLs), Autoinducer Peptides (AIPs), and Autoinducer-2. Beside these, additional signalling molecules such as streptomyces butyrolactones, diffusible signal factors, cyclic diguanylate (c-di-GMP), pseudomonas quinolone signal (PQS), ydroxyl-palmitic acid methyl ester (PAME) and diketopiperazines (DKPs), are present in some bacteria as depicted in Figure.1 [4–13].

Acyl-Homoserine Lactones (AHLs) are signalling molecules commonly present in gram-negative bacteria. They consist of a lactone ring with an attached acyl chain of varying lengths. AHLs are produced by an enzyme called AHL synthase which is encoded by gene LuxI. AHL dependent pathways rely on three main factors: AHLs, synthesizing enzymes, AHLs and regulatory protein that regulates the surrounding concentration of AHLs [14–15]. P.aeruginosa, Burkholderia cepacia, and P. putida are gram negative bacteria’s that communicate through AHLs. Exceptionally, Exiguobacterium sp., a gram-positive bacterium also communicates through AHLs [16–18].

Gram-positive bacteria use Autoinducer Peptides (AIPs), a type of signalling molecule. These are short post-translationally modified peptides secreted by the bacteria. Autoinducing peptides such as

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thiolactones, oligopeptides for instance. Autoinducers interact with transcriptional regulators either directly or by activating a sensor kinase [14]. C. perfringens, S. aureus, Clostridium difficile, Lactobacillus plantarum, C. botulinum, and Enterococcus faecalis in Gram positive bacteria communicate using quorum sensing molecules called AIPs [19–21]. Additionally, it is interesting to note that activated methyl cycle produces autoinducer-2 which is found in both the species for interspecies communication purpose. Vibrio cholerae, V.harveyi , S.typhi , E. coli and Deinococcus radiodurans use AI-2 signalling pathway [22].

Bacterial cell densities determine the concentration level of these signal molecules. As bacterial population increases its signalling molecule concentrations also go up with time. Once a threshold concentration is attained they bind to their cognate receptors leading to induction of specific gene expression patterns within the bacterial populations resulting into coordinated behaviour such as biofilm formation, virulence factor production, sporulation, bioluminescence and antibiotic production [23–25].

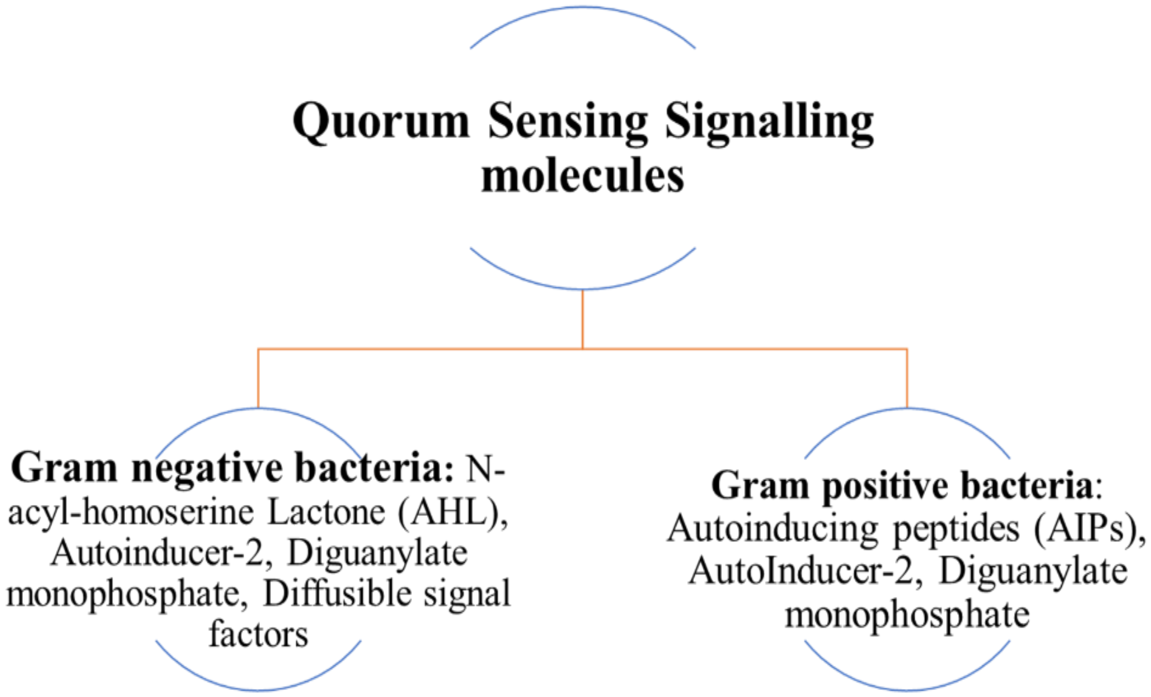

Figure 1. Quorum sensing signalling molecules

Role of Qs in Biological Wastewater Treatment

Quorum sensing has emerged as a vital factor in understanding and optimizing biological wastewater treatment processes. Through the study of QS mechanisms, researchers have gained insights into the behaviour of bacteria in wastewater treatment systems and their responses to changing environmental conditions. Microorganisms are widely used in wastewater treatment for their property to degrade pollutants. They uptake the contaminants present in the environment, metabolize them and used them as energy source [26]. Growth of microorganism results in the formation of dense microbial population in the form of biofilms, granules, and flocs. Biofilms plays both positive and negative role in wastewater bioremediation [27]. Various reactors like moving bed biofilm reactor, biological contact oxidation tank, trickling filters uses biofilm for the waste water treatment effectively eliminating organic and nitrogenous pollutants while generating minimal sludge. Additionally, immobilized cells within the biofilm produces enzymes important for bioremediation. In contrast, biofilm and EPS secreted by the bacteria accumulates on the membrane surface causing membrane biofouling [13–28]. Controlling biofilm production in wastewater treatment is vital as uncontrolled biofilm formation leads to membrane biofouling. Excessive biofilm formation reduces the efficiency of the treatment process. It can lead to clogging of pipes, filters, and other equipment in wastewater treatment plants. This can reduce the flow rate and efficiency of treatment processes, resulting in decreased treatment capacity, decreased water quality and increased operational costs [2].

QUORUM SENSING INHIBITION METHODS

Interfering with the quorum sensing mechanism results in the inhibition of quorum sensing. This interference aims to prevent or reduce bacterial coordination and communication, thereby inhibiting the expression of genes involved in virulence factor production, biofilm formation, and antibiotic resistance [29]. There are several strategies employed to inhibit quorum sensing: Quorum quenching, enzymatic degradation, Quorum sensing inhibitors (QSIs), Degradation by ROS as shown in Figure. 2.

Quorum Quenching Bacteria

Some bacterial strains have the ability to produce enzymes known as quorum quenching enzymes, which degrade or modify signaling molecules, thereby inhibiting quorum sensing [30]. Naturally occurring quorum quenching bacteria that has been reported are Bacillus methylotrophicus sp. WY, Rhodococcus sp. BH4, Penicillium restrictum CBS 367.48, Bacillus licheniformis T-1, and Pseudomonas sp. HS-18 [31–32]. Muricauda olearia Th120 produce enzyme MomL lactonase which inhibits AHL thus inhibiting virulence [33]. Listeria grayi (HEMM-2) was isolated and applied for the degradation of AHLs. This QQ bacteria degraded the AHLs regardless of their length and was able to downregulate the gene system of Pseudomonas aeruginosa which resulted in inhibiting biofilm formation by 53-75% [34]. Bacillus thuringiensis LZX01, a novel QQ bacteria, secretes intracellular lactonases which helps in delayed biofouling by degrading 90% of C8-HSL within 30 min at a concentration of 200ng/ml [35]. Employing genetic engineering techniques for producing new QQ strain with more promising degradation. AHL producing QS bacteria in coordination with AHL degrading QQ bacteria has been reported to be present in activated sludge at the same time [32].

Quorum Quenching Enzyme

Quorum quenching method consists of enzymatic degradation of signaling molecules produced by bacteria. Enzymes such as deaminase, lactonase and decarboxylase have the ability to utilize acyl-homoserine lactones (AHLs) as a substrate, thus disrupting quorum sensing [31–32].

Quorum sensing inhibitors (QSIs)

This method involves disruption of Quorum sensing receptors with the help of QS inhibitory compounds. Examples of such inhibitors are gingerol, vanillin, furanone, homoserine lactone-like TGK series, 3-amino2-oxazolidinone YXL-13, cladodionen, etc [30]. Phytochemicals are also reported to be a good QS inhibitor like Rhamnolipid, Pyocyanin, Piericidin A, Taxifolin, Eriodictyol, Quercetin, Catechin, Anthocyanin-cyanin, Rosmarinic acid, obacunone, Ichangin, Isolimonic acid, Carvacrol, Malvidin, Malabaricone C [36]. QSI have a potential to prevent biofilm formation and control membrane biofouling in wastewater treatment systems. It has been reported that application of vanillin on the RO membrane decreases the biofilm formation by 45% and tetrachlorosalicylanilide inhibits biofilm formation by 50% [32]. Chen et al. developed a novel Seawater reverse osmosis membrane for desalination process by incorporating methyl anthranilate (MA), a QS inhibitor into the membrane. This membrane resulted in 80% decrease of dominant bacterias like Shewanella, Colwellia, Arcobacter and their metabolic products [37]. Li et al utilized quercetin as a inhibitor, they reported that quercetin was able to inhibit AI-2 in E.coli and AGR in S.aureus with a inhibition rate of 53.1% and 57.4% respectively [38].

Reactive Oxygen Species (ROS): ROS (superoxide and hydroxyl radicals) are recently used methods for QS inhibition. ROS produced through UV-TiO_2 photocatalysis deactivated AI-2 released by E. coli and decreased biomass by 42.6% [39].

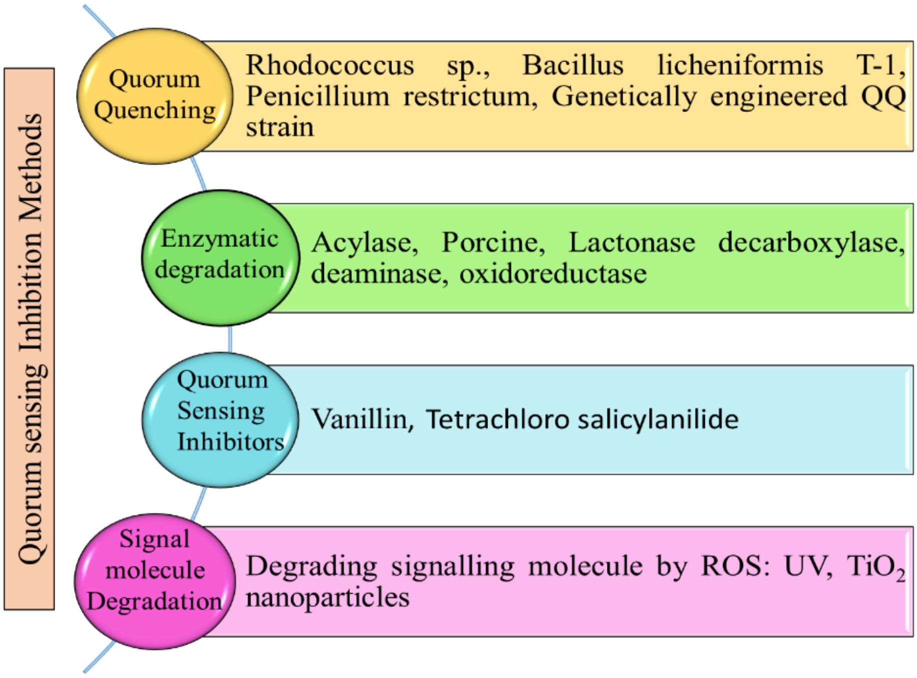

Figure 2. Methods for Quorum Sensing Inhibition

Strategies To Control Biofouling

QS Mechanisms Can Be Broadly Classified into Two Main Types: homoserine lactone (AHL)-based systems and AIPs based systems [30–40]. The AHLs signalling molecule, primarily utilized in gram-negative bacteria, is regulated by LuxR/I gene system [41]. AHLs are synthesized by the LuxI gene in the presence of Autoinducer Synthase. These lipophilic molecules are capable of crossing the bacterial membrane. Once the AHL concentration in the extracellular matrix reaches a threshold, it enters into the cell and binds to its receptor. This binding trigger upregulation of the LuxR/I system, activating gene expression responsible for virulence factors [15–28–36]. On the other hand, Autoinducing Peptides (AIPs) serve as signaling molecules in gram-negative bacteria. AIPs are produced by gene system in the presence of Autoinducing Peptide Synthase. These peptides are then transported outside the cell into the extracellular matrix through ATP-binding cassette (ABC) transporter. Once the concentration of AIPs reaches a threshold, they get transported into the cell through histidine kinase. Subsequently, they undergo phosphorylation, activating the response regulator, which then binds to DNA, initiating gene expression associated with virulence [30–36–40–42]. AI-2 Based Systems are found in both Gram-negative and Gram-positive bacteria. AI-2 is synthesized by the LuxS enzyme and helps in inter- and intraspecies communications. This enables different bacterial species to coordinate their behaviours based on AI-2 signals, even in mixed microbial communities [15]. Additionally, three more *QS systems are identified in gram-negative bacteria*: diffusible signal factor (DSF) in Xanthomonas campestris was reported to be responsible for biofilm formation, production of extracellular enzymes, survival, Autoinducer 3 in E.coli, and Pseudomonas quinolone signal (PQS) in *P. aeruginosa*: (Las and rhl system) [40].

Disrupting QS Can Be Achieved Through Various Strategies: i) Inhibition of AI synthase, ii) Autoinducer inhibition or degradation, iii) Interfering with signal receptors, iv) Interfere with response regulators as shown in Figure. 3 [30–40]. Inhibition of AI synthase: One strategy for inhibiting quorum sensing is to interfere with the synthesis of autoinducers. This can be achieved by using small molecules or compounds that inhibit the enzymes responsible for autoinducer production. By limiting the availability of autoinducers, the bacteria are unable to reach the required population density to trigger biofilm formation. LuxI uses SAM and Acyl-ACP as a precursor for AHL synthesis. It has been reported that analogs of AHL such as L/D-S-adenosylhomocysteine, sinefungin, and butylryl-Sadenosylmethionine (butylryl-SAM) were able to inhibit AHL synthesis [40–42]. Brominated furanones have been identified as a inhibitor of LuxS gene which is responsible for AI-2 synthesis [43].

Autoinducer Inhibition or Degradation: Another approach is to directly inactivate or sequester the autoinducers, preventing them from binding to their respective receptors. This can be achieved through the use of enzymes or molecules that degrade or bind to the autoinducers, rendering them ineffective. Lactonases, acylases and oxidoreductase was found to modify or inactivate AHL [44].

Interfering With Signal Receptors: Targeting the receptors involved in quorum sensing is also an effective strategy. Small molecules or peptides can be used to competitively bind to the receptors, preventing the autoinducers from binding and triggering the signaling cascade[30]. By interfering with receptor function, the bacteria are unable to receive the quorum sensing signals, inhibiting biofilm formation. Furanone isolated from red alga Delisea pulchra was able to target S. liquefaciens MG1, V. fischeri, V. harveyi, and Serratia ficaria [45–46]. Vanillin interferes with the receptor of AHL in Aeromonas hydrophila [47].

Interfere With Response Regulators: In addition to targeting the autoinducers and receptors, disrupting the downstream signaling pathways can be a viable strategy. By inhibiting the intracellular signaling cascades triggered by the quorum sensing signals, the bacteria are unable to activate the genes and behaviors associated with biofilm formation. This can be achieved using compounds or molecules that target specific proteins or enzymes involved in these pathways.

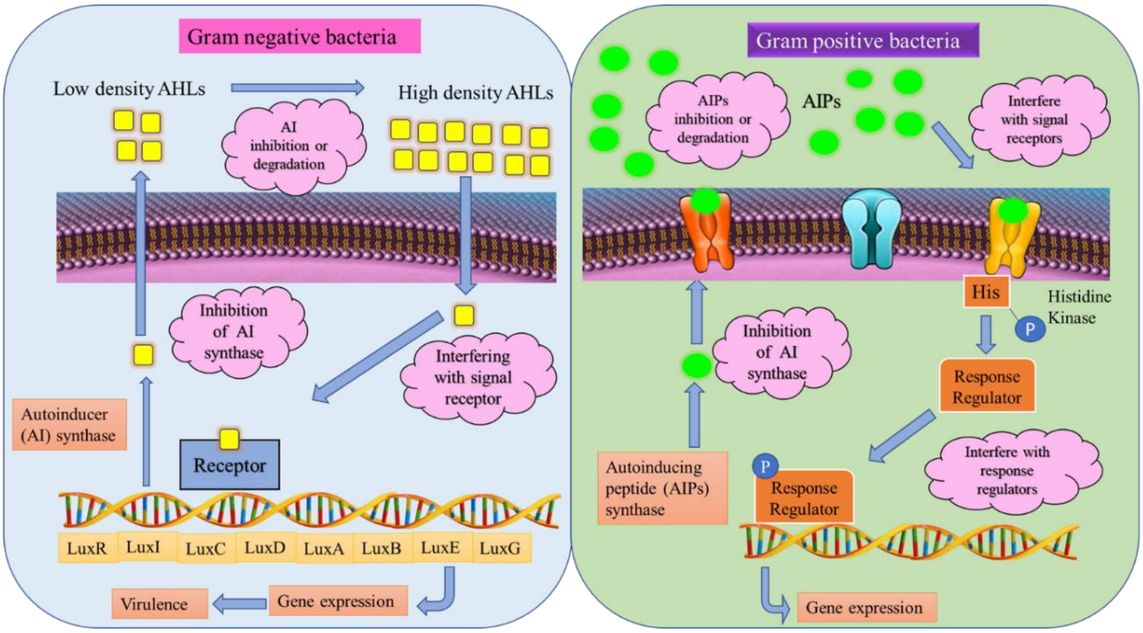

Figure 3. Quorum sensing pathway and strategies to control biofouling.

Conclusion And Future Perspective

Membrane based bioreactors are efficient technology for the treatment of wastewater. It has various advantages like, smaller footprints, high quality water. Some specific applications include: Reverse Osmosis Systems, membrane bioreactors, nanofiltration and ultrafiltration systems. But, it has one major drawback of membrane biofouling leading to decreased water flux, operational costs and increased energy consumption. It is caused by accumulation of microorganism on the membranous surface, in some cases it is combined with organic and inorganic matter. Various physical, chemical and biologicals methods are used to prevent the formation of biofilms. On the basis on various researches that are focused on the role of Quorum sensing in regulation of virulence factors and biofilm formation. Studies has focused on inhibition of quorum sensing to control membrane biofouling.

Comprehending quorum sensing mechanisms facilitates the development of strategies to suppress microbial biofilm formation on membranes. By preventing or reducing the formation of biofilms on membrane surfaces, the efficiency and lifespan of membranes used in water treatment can be improved. So, this approach can be used as a promising strategy for controlling membrane biofouling in membrane-based reactors.

Competing Interest and Funding

The authors declare that they have no conflict of interest and no funding was received to assist with the preparation of this manuscript.

Author's Contribution Statement

All authors equally contributed to the study conception and design. Material preparation, data collection and analysis were performed by Priyanka Kakkar. The first draft of the manuscript was written by Prof. Neeraj Wadhwa. All authors read and approved the final manuscript.

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