

## Self-Assembling Bisbenzimidazole Derivatives: A Pioneering Approach to Bacterial Flocculation

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Bacterial flocculation is a biological process that has gathered increasing attention in recent years due to its diverse applications, spanning from wastewater treatment to biodegradation or biocatalytic processes. During this phenomenon, bacteria aggregate into visible flocs, facilitating the straightforward recovery of cells. While this process can occur naturally, flocculating agents are generally used to improve the flocculation efficiency<sup>[1]</sup>. The primary mechanism of action of these agents relies on electrostatic interactions between the bacterial membrane (negatively charged) and the flocculating agent (positively charged)<sup>[2]</sup>. However, this specific mechanism of action, coupled with elevated costs and toxicity, presents challenges that hinder the widespread industrial application of current flocculating agents<sup>[1]</sup>.

In recent investigations, our research team has highlighted the intriguing biological activity exhibited by specific bisbenzimidazole derivatives. These compounds display substantial efficacy against both Gram-positive and Gram-negative bacterial strains, as evidenced by the formation of flocs within minutes when the bacterial suspension is exposed to a low concentration of a bisbenzimidazole compound. This phenomenon indicates a bacterial flocculation process initiated by these derivatives.

Since the structure of our compounds shows no indications of a conventional flocculation mechanism (no presence of positive charge in the molecule), we opted to conduct additional investigations. Initially, a range of microbiological studies (*e.g.* microscopic examinations, biological characterizations of the flocs) revealed that the observed flocculation did not emerge from a biological process but rather from physicochemical factors. The literature widely acknowledges that numerous heterocyclic structures have the ability to self-assemble in solution, forming supramolecular arrangements<sup>[3]</sup>. Through AFM studies, we successfully illustrated the compounds' ability to create fibre networks in solution through a molecular self-assembly process. Consequently, we were able to establish a correlation between the formation of this network and the onset of the flocculation phenomenon, this offering a new class of rapid and effective bacterial flocculating agents.

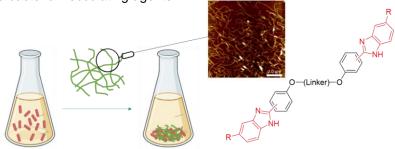


Figure 1: Bacterial flocculation is induced by bisbenzimidazole derivatives' self-assembly.

## References

[1] Ojima, Y., Azuma, M. & Taya, M., World Journal of Microbiology and Biotechnology (2018) 34, 185.

- [2] Yang, Z. et al., Environmental Science & Technology (2014) 48, 6867–6873.
- [3] Palermo, V. & Samorì, P., Angewandte Chemie International Edition (2007) 46, 4428–4432.