Fluorescent probe: A novel visualization tool for rapidly assessing the health risk of organisms exposed to microcystins

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Microcystins (MCs) are a type of monocyclic cyanotoxins and commonly found in eutrophic waters where cyanobacteria bloom outbreaks.[1] MCs continuously attract research interest due to their potent toxicity and wide distribution. Hundreds of MC subtypes have been successively identified over the past decades, and most isoforms are capable to maintain their chemical stability in the environment.[2] To prevent public risks, 1 μg/L was suggested to be the tolerable daily intake of MCs in drinking water. The occurrence of harmful cyanobacteria blooms has been reported multiple times globally. This situation indicates that aquatic plants, animals, and even humans may be under the potential threat of environmental exposure to MCs, particularly under expanding climate changes and aquatic eutrophication. Therefore, a simple method for rapidly assessing the health risks of organisms exposed to MCs is needed.

The health risks of animals and humans associated with exposure to MCs have been observed since the end of the 20th century. In 1998, Codd et al reported the first poisoning incident caused by acute exposure of MCs. More than 60 patients died because the water used for dialysis was contaminated with cyanobacteria.[3] In recent studies, MCs have been detected in various aquatic organisms and the serum of fishers after long-term environmental exposure. The accumulation of MCs *in vivo* not only leads to multiple toxicities, but also may increase the risk of cancer. Fluorescent probes, as a visualization tool, have been frequently used and recognized in the field of environmental pollutant detection, food safety, and biomedicine.[4] This novel technology not only has the advantages of high sensitivity, low cost, and rapidly response, but also shows the strong ability of in-situ detection and real-time monitoring.[5] Therefore, we proposed that fluorescent probe technology could be a novel visualization tool to rapidly evaluate the health risks of organisms exposed to MCs.

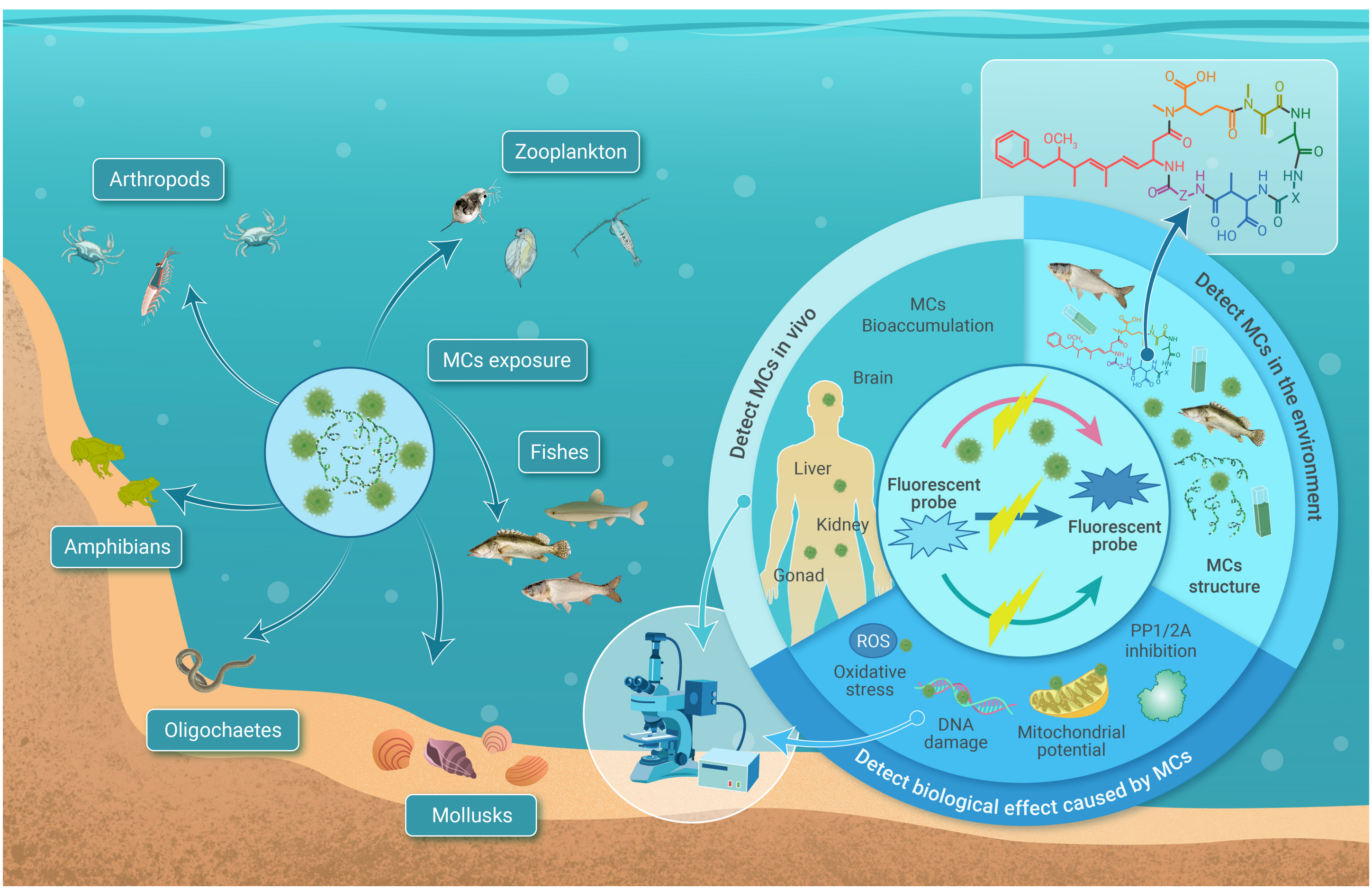

Figure 1. Perspective of using fluorescent probe to assess the health risk of organisms exposed to MCs The exposure of MCs poses a serious health threat to various organisms in the environment. Fluorescent probes are expected to detect the exposure risks of MCs in three directions.

The rise of probes in the environmental detection field shown the great potential of using probes to quickly assess the health risks of organisms exposed to MCs. Microcystin-LR (MC-LR), as a representative MCs, has become the preferred choice for research due to its strongest toxicity and widest distribution. In 2022, our research group first developed a fluorescent probe MC-BDKZ, which has achieved the detection of MC-LR in water samples and cells. Subsequently, inspired by the unique monocyclic configuration of MCs, triphenylamine and tetrastyrene groups have also been explored for constructing probes, which recognize MC-LR through the CH−π interaction between aromatic rings and amino acids. Moreover, the study based on fluorescent probes to assess the environmental exposure hazards of toxins to plants and zebrafish has been reported. Therefore, compelling evidences suggest that fluorescent probes could be an effective tool for evaluating the health risks associated with MCs.

Three research directions for fluorescent probes were proposed to comprehensively evaluate the health risks of MCs exposure (Figure 1). (1) Specific MC probes should be developed to directly detect MCs in the environment. In natural lakes, cyanobacteria not only produce and release MCs, but also produce various other types of cyanobacterial toxins. Moreover, the water environment itself is a complex system. Therefore, the ideal MC probes should have the ability to specifically detect MCs and should not respond to other types of compounds. Developing fluorescent probes that can specifically recognize MCs is crucial for practical applications. The test strips and test kits based on highly sensitive probes for rapid detection of MCs in the environment have great economic value and social applications. (2) Fluorescent probes for detecting the accumulation of MCs in organisms should be constructed. MCs, after being released into the water environment, can be transmitted through the food chain and then accumulate in various animals. MC probes are expected to detect the presence of MCs in organisms and then indicate the accurate content of MCs through the difference in fluorescence signals. This type of probes will have significant performance in studying the accumulation, migration, and transformation of MCs. (3) Probes should be used to effectively monitor the biological damage caused by MCs. The normal life and physiological processes in organisms are in a dynamic equilibrium state, as regulated by multiple biological factors. MCs, after entering the body, will cause oxidative stress and protein phosphatase inhibition and then induce a series of existing homeostasis imbalances, which play key roles in the occurrence of mitochondrial autophagy, cell apoptosis, and related diseases. Detecting the steady-state fluctuations will reflect the biological impact caused by MCs.

Fluorescent probe technology has exhibited a great prospect for visualizing the risks of organisms exposed to MCs. However, many challenges still need to be addressed: (1) Research on developing MC probes currently remains in the preliminary stage. Constructing specific MC probes based on traditional recognition sites, such as Adda, carboxyl group, guanidinium group, and unsaturated group, is difficult. Considering the special monocycle configuration of MCs composed of seven amino acids, the structure of pyridine nitrogen, polybenzene ring, and long alkyl chain were deducted to have great potential for constructing MC probes. (2) Direct in-situ detection and real-time monitoring of MCs in organisms are difficult to conduct. The applicable samples of existing methods for detecting MCs *in vivo* are mainly cell or tissue homogenates, rather than living animals. The fluorescent probes for detecting MCs in organisms should have good tissue penetration. The near-infrared (NIR) fluorescent probes with high excitation and emission exhibit strong resistance to biological background. NIR MC probes with excellent tissue penetration should be designed preferentially to achieve the expected experiment results. (3) Suitable markers of biological damage resulting from exposure to MCs should be selected. Biomolecules and signaling molecules that regulate physiological processes may be abnormal or overexpressed after the body is stimulated. Combined with the toxicity mechanism of MCs, the reactive oxygen species level, protein phosphatase activity, and mitochondrial membrane potential can be selected as the markers, and the corresponding detection probes can be further explored. In addition, the toxicity and anti-interference ability of the developed probes need to be considered.

The environmental pollution problem of MCs has become increasingly serious with the frequent occurrence of water eutrophication. MCs released by cyanobacteria exhibit strong toxicity and pose a potential health threat to organisms. This editorial suggests that fluorescent probe technology can be used as a new detection method to comprehensively evaluate the health risks of MCs. This approach visualizes and detects MCs in the environment, MCs *in vivo*, and the disease impact of MCs on biological systems. The above-mentioned comments encourage that specific MC probes can be developed to directly detect MCs in the environment. Moreover, the accumulation and toxic effects of MCs *in vivo* could be indicated through different fluorescent probes. Constructing fluorescent probes for rapid in situ detection of MC accumulation and the impact of MCs on organisms can help comprehensively analyze the health risks of MCs and may provide direct evidence for the impact of MCs on human health under natural poisoning conditions. This editorial presents a new research viewpoint for applying fluorescent probe technology in ecotoxicology research on MCs. The application of using fluorescent probes to evaluate the health risks of exposure to MCs is an innovative exploration that requires overcoming many difficulties. To protect public health, more probes need to be developed to quickly identify the potential environmental hazards of MC exposure threats.

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DECLARATION OF INTERESTS

The authors declare no competing interests.