# Novel Detection Technology by Cascade Enzymatic Reactions

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**Abstract**

Two-component flavin-dependent monooxygenases are enzymes that can catalyze a wide variety of reactions. Many of these can be applied in detection and bioreporter applications. These enzymes require two protein components; one is a flavin reductase and another is a flavin-dependent monooxygenase which can catalyze a single-atom oxygen insertion. Depending on their active site architecture and reaction specificity, these oxygenase components can also catalyze extra reactions in addition to the oxygen incorporation. Our group has studied the reaction of a flavin-dependent monooxygenase HadA which catalyzes dehalogenation and denitration of 4-halogenated and 4-nitro phenols to generate *p*-benzoquinone. As *p*-benzoquinone from the HadA reaction can react with D-cysteine to generate D-luciferin which is a substrate for firefly luciferase, the presence of p-halogenated and p-nitro phenols can be converted to light emission using reactions of HadA and firefly luciferase. Because halogenated and nitro phenols are used widely as agro- and industrial chemicals, technology that can detect their accumulation and widespread in the environment will help in prevention of their contamination in food and fresh water resources. Another two-component flavin-dependent monooxygenase system that is useful for detection application is bacterial luciferase which catalyzes a light emitting reaction with lmax 490 nm using reduced flavin and long chain aldehyde and molecular oxygen as substrates to yield oxidized flavin, carboxylic acid and water. As substrates of the Lux reaction are cheaper than substrates of other bioluminescence systems, Lux is useful as a bioreporter system for molecular biology research. We have developed Lux as an alternative bioreporter to be used in eukaryotic systems using approach of enzyme engineering. We have also developed a self-sufficient auto-luminous system using flavin and carboxylic acid reductases. The cost and efficiency of the system were also optimized using analogues of NADH which is a reductant for reductase reactions.

**Keywords:** Cascade reaction, Halophenol and Nitrophenol detection, Bacterial luciferase, Bioluminescence, Bioreporter gene

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