CHAPTER II
LITERATURE REVIEW

2.1 ESKAPE pathogens

The ESKAPE pathogens are a group of six bacterial pathogens known for their high resistance to antibiotics, making them major concerns into public health. The term "ESKAPE" is an acronym for the following bacteria: E stand for *Enterococcus faecium*, S stand for *Staphylococcus aureus*, K stand for *Klebsiella pneumoniae*, A stand for *Acinetobacter baumannii*, P stand for *Pseudomonas aeruginosa*, and E stand for *Enterobacter spp.* These pathogens are frequently linked to many clinical syndromes such as bloodstream infections, urinary tract infections (UTI), pneumonia, and other healthcare-associated Infections (HAIs) (Luo et al., 2024). Their capacity to develop resistance to several antibiotics, including drug uptake limitation, drug target site modifications, enzyme-mediated drug inactivation, and active drug efflux, make them were identified as critical multidrug-resistant bacteria (Singh et al., 2024). Table 2.1 shows the overview of ESKAPE pathogens including their antibiotic resistance and major clinical syndrome.

Table 2.1 Overview of ESKAPE pathogens (Luo et al., 2024; Miller and Arias, 2024).

Bacterial species	Antibiotic resistance	Major clinical syndromes
Enterococcus spp.	Vancomycin, linezolid, ciprofloxacin, nitrofurantoin	Bloodstream infection, infective endocarditis, intra-abdominal infection, UTI
S. aureus	Methicillin, oxacillin, penicillin	Bloodstream infection, infective endocarditis, ABSSSI, CAP, HAP/VAP, bone and joint infection
K. pneumoniae	Carbapenem, cephalosporin, aminoglycoside, fluoroquinolone, polymyxin, tigecycline	Bloodstream infection, UTI, CAP, HAP/VAP, intra-abdominal infection
A. baumannii	Carbapenem, β-lactams, aminoglycoside, fluoroquinolone	HAP/VAP, bloodstream infection, UTI
P. aeruginosa	Carbapenems, piperacillin-tazobactam, fosfomycin	HAP/VAP, bloodstream infection, UTI
Enterobacter spp.	Carbapenems, β-lactams, polymyxins, cefotaxime	Bloodstream infection, UTI, HAP/VAP, intra-abdominal infection

ABSSSI, acute bacterial skin and skin structure infection; CAP, community-acquired pneumonia; HAP/VAP, health-care-associated pneumonia/ventilator-associated pneumonia; UTI, urinary tract infection

2.2 Structure and Mechanism of Nitroreductases (NTRs)

Nitroreductases (NTRs) are a family of flavoenzymes expressed in bacteria and a few eukaryotes (Boddu et al., 2021). These enzymes can reduce a variety of nitro-containing compounds into amino groups by the reduced flavin cofactor at the active site, which is reduced by NADH or NADPH (Haynes et al., 2002).

Nitroreductase can be classified into two functional classes based on their sensitivities to oxygen: oxygen-insensitive (Type I) and oxygen-sensitive (Type II) reductases. There are two possible ways for NTR enzymes to reduce nitro groups, as seen in Figure 3. Type I nitroreductase uses a two-electron transfer mechanism to reduce the nitro group into nitroso, hydroxylamine, and amine. Nitro compounds, hydroxylamine, and amine metabolites are stable, whereas nitroso intermediate is unstable because the second and third two-electron transfer is faster than the first two-electron reduction. Consequently, the nitroso intermediate can react with biomolecules, leading to the formation of toxic and mutagenic products. Conversely, Type II nitroreductase uses a single electron transfer mechanism to carry out reduction reactions, resulting in an unstable nitro radical anion that reoxidizes to a starting nitro compound and produces a superoxide anion with a futile cycle under aerobic conditions. In the absence of oxygen, two nitroradical anions create the initial nitro compound and the nitroso derivative. It is thought that this is how nitroso compounds, which are observed in biological systems, can be formed (Rice et al., 2021). In *Escherichia coli,* there are two genes of oxygen-insensitive (Type I) nitroreductase, NfsA and NfsB. NfsA uses NADPH as an electron donor, whereas NfsB can use either NADH or NADPH as a source of reducing equivalents (Whiteway et al., 1998).

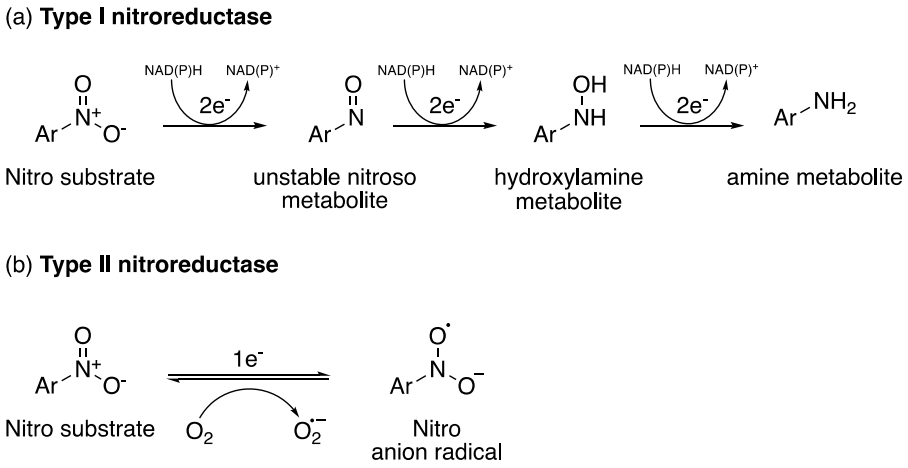

Figure 2.1 Reduction of a nitro group catalyzed by Type I (a) and Type II (b) nitroreductases, respectively. (Modified from Pimviriyakul et al., 2023).

2.3 Detection of nitroreductase activity

In pathogenic bacteria, Nitroreductases can be involved in the inactivation of antibiotics or the detoxification of nitro-containing compounds by a redox process that permanently degrades or neutralizes antibiotics (Thomas and Gwenin, 2021). It is also used in the activation of nitro-containing prodrugs such as Tretazicar (CB1954), which is an anticancer prodrug, and an antibacterial diazeniumdiolate prodrug (Hibbard and Reynolds, 2019; P. F. Searle et al., 2004). Detecting nitroreductase activity in pathogenic bacteria can provide insights into their virulence mechanisms and aid in the development of novel antimicrobial strategies. In cancer cells, it was presumed that hypoxia can promote overexpression of the intracellular reductases such as NTR (Nitroreductase), AzoR (Azoreductase) and DTD (DT-diaphorase). Therefore, nitroreductase is attractive for evaluating the regions with low oxygen levels in tumors, which are generally termed as hypoxia (Janczy-Cempa et al., 2021; Kumari et al., 2019).

Recently, many researchers have developed methods for detecting nitroreductase activity in bacteria or hypoxic cells by turn-on-off fluorescence probes, which could be promising tool for detecting pathogen infection or cancer cells. Because nitroreductases catalyze the reduction of nitro-containing compounds in the presence of NAD(P)H, the development of nitroreductase turn-on fluorescence probe has gained much attention. Wang and colleagues developed a benzoindole-based fluorescent

probe, NFP-NTR, for the detection of nitroreductase (NTR). This probe selectively reacts with NTR in the presence of NADH, facilitating the cleavage of the *p*-nitrobenzyl ether bond and generating a fluorescence signal. The proposed sensing mechanism of NFP-NTR for NTR is depicted in Figure 2.2, where NTR catalyzes the reduction of the electron-withdrawing nitro group (–NO_2) to an electron-donating amino group (–NH2). This transformation induces self-immolation ether cleavage, ultimately releasing the fluorophore NFP-1, resulting in a significant increase in fluorescence intensity(Wang et al., 2023). Additionally, NFP-NTR exhibited a detection limit of 17 ng/mL and demonstrated exceptional selectivity and sensitivity. The probe has also proven effective for imaging live HeLa cells in hypoxic conditions with minimal cytotoxicity, highlighting its potential for further biological applications related to NTR activity.

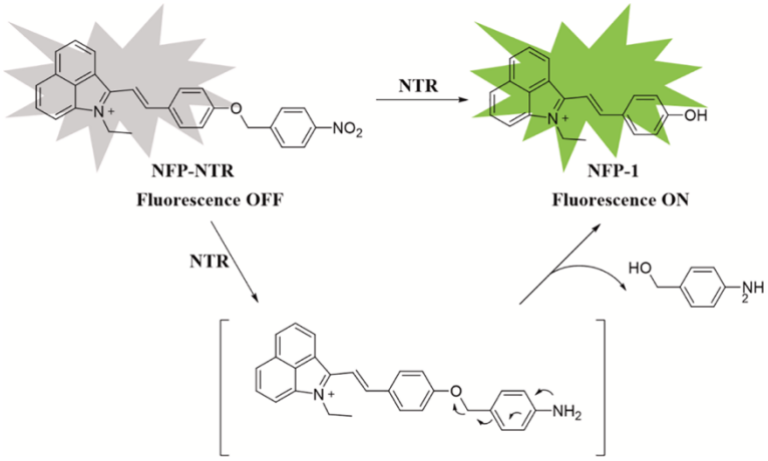

Figure 2.2 Proposed mechanism of the fluorescence signal turn-on of probe NFP-NTR catalyzed by NTR (Wang et al., 2022).

In 2023, Yan and colleagues introduced TCF-Nitro, a red-emitting NTR sensor with a long-wavelength emission at 560 nm, designed for detecting NTR activity in bacteria. This sensor is based on a modified **2**2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) scaffold, incorporating an NTR-responsive nitro locking group (Figure 2.3). TCF-Nitro enables the detection of NTR and facilitates the monitoring of NTR levels in *E. coli* as well as other clinically significant bacterial species (Yan et al., 2023).

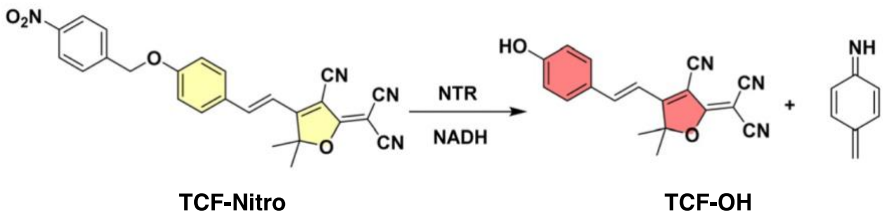

Figure 2.3 NTR catalyzes the reduction of the nitro group in TCF-Nitro to an amine, initiating a fluorescence "turn-on" response as a result of TCF-OH release (Yan et al., 2023).

2.4 Fluorescence

Fluorescence is the process in which a substance absorbs light or other electromagnetic radiation at a specific wavelength and subsequently emits light at a longer wavelength. The molecules that show their fluorescent properties are called fluorophores. The emitted light is typically in the visible spectrum, even if the absorbed light is in the ultraviolet (UV) range (Lichtman and Conchello, 2005) . The Jablonski diagram illustates how a fluorophore emits light after absorbing the light (Figure 2.4). When the fluorophore do not absorb energy or absorb excitation light, electrons are confined in the ground state **(S_0).** After electrons were excited by interacting with a wavelength of photons or excitation wavelength (λ_{ex}) as shown in blue, the interaction between photons and fluorophores promoted the transition from the ground state (S_0) to higher energy states. Electrons at the higher excited states move to the S_1 state through heat emission and other non-radiative processes. When electrons fall from S_1 to S_0, the photons of light are released as shown in the green arrow, which makes the molecules fluorescent (Llères et al., 2007). The fluorescence wavelength is also called as emission wavelength (λ_{em}).

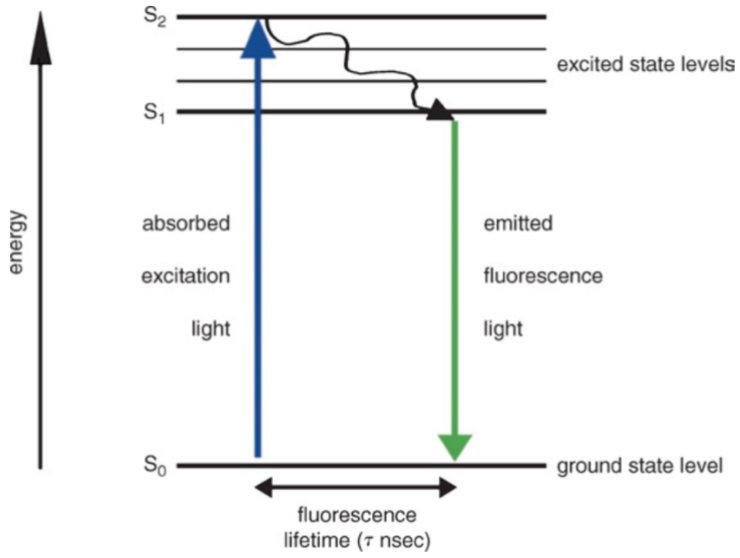

Figure 2.4 The Jablonski diagram. (Llères et al., 2007)

2.5 References

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