CHAPTER V
CONCLUSIONS

In summary, we designed a novel fluorescence turn-on probe IND-NO_2 (NTR probe) to detect nitroreductase (NTR) in bacteria cells. Synthesis of the IND-NO_2 probe was completed in a few steps. To test its NTR-activated property, *Ec*NfsB was used as a model enzyme. The enzymatic studies showed that *Ec*NfsB catalyzes the reduction of IND-NO_2 in the presence of NADH to generate IND-OH, which was characterized by fluorescence spectroscopy and HPLC analysis. The fluorescence turn-on signal resulted from IND-OH with the fluorescence maximum at 564 nm (λ_{ex} = 520 nm). In addition, IND-OH displayed pink color, which can be observed by naked eyes. The photophysical properties of IND-OH made it promising in practical applications. Furthermore, the reduction of IND-NO_2 is specific to the NTR-catalyzed reaction while all tested biological reductant molecules did not reduce the probe. The LOD value for *Ec*NfsB was determined as 6.21 nM, which is lower than other *E. coli* NTR probes. In bacteria tests, the IND-NO_2 probe was incubated with the in-house bacteria, *E. coli, S. aureus,* and *P. aeruginosa* representing ESKAPE pathogens, to show the higher fluorescence signals compared with the decreasing signals in reaction of the same bacteria containing an NTR inhibitor, suggesting the NTR-catalyzed turn-on signal was resiponsible for the change in fluorescence. Overall, the designed probe was demonstrated as a potential tool for identifying ESKAPE pathogen infections.