

## Abstract

Light-producing protein enzymes such as luciferase play important roles in both applied and basic research. In this study, we used an *in vitro* selection to isolate deoxyribozymes that catalyze a chemiluminescent reaction by dephosphorylation of the commercial substrate CDP-Star. One of the most active variants, named Supernova, was further improved and characterized using a combination of random mutagenesis, *in vitro* reselection, high-throughput sequencing, comparative sequence analysis, and optimization of reaction conditions. Supernova produces light up to 6,500-fold more efficiently than the background reaction and folds into an unusual triple-helical structure. Moreover, we characterized in detail the buffer requirements including pH, the effect of various ions, substrate and Supernova concentrations, and the presence of crowding agents. Finally, we showed that Supernova can be turned into an allosteric sensor by rational design. We anticipate that this deoxyribozyme can be used as the signaling component in light-producing allosteric deoxyribozyme sensors that respond to a wide variety of stimuli and will complement existing methods that utilize radioactive, fluorescent, and colorimetric readouts.