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Reactive oxygen species (ROS) are reactive molecules and free radicals derived from molecular oxygen. ROS are the cause of oxidative stress in cells, and can damage cellular components such as DNA, lipids, and proteins. Recent studies have shown that ROS play an important role in fungicidal action of antifungals; however, the specific action causing cell death is unknown. In addition, it has been proposed that a common mechanism exists for bactericidal antibiotics, including the production of ROS. However, the mechanism and extent to which ROS are involved is widely debated. Therefore, further studies must be conducted to determine the specific relationship between antimicrobials and ROS production. This is particularly important because of the growing public health concerns regarding antibiotic and antifungal resistance. Here we present two novel and genetically encoded redox-sensitive fluorescent proteins developed by our lab to detect cellular ROS. These fluorescent proteins are redox-sensitive through the substitution of cysteines at certain surface exposed residues which allow the formation of disulfide bonds in oxidizing environments. This disulfide bond in turn distorts the chromophore of the protein, altering its fluorescent properties. Our novel biosensors "royRFP" and "roxyYFP" are engineered variants of RFP (red fluorescent protein) and YFP (yellow fluorescent protein), respectively. Initial data show that these redox-sensitive fluorescent reporters may be reliable and effective tools for detecting cellular ROS. We anticipate these biosensors to provide new methods for studying antimicrobial resistance in *Escherichia coli*, and *Candida albicans*, as well as a screening platform for novel antibiotics and antifungals.