Title: Engineering prokaryotic repressors for optical sensing of neurotransmitters

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Abstract: As chemical transmission plays a critical role in neural communication, there is a growing need for tools that can report on the dynamics of neurotransmitters *in vivo*. Here we explored the use of prokaryotic transcriptional repressors, specifically TetR family repressors, for sensing melatonin and N-acetylserotonin (NAS). The TetR family of repressors is a highly diverse family of genetic regulators that share the same general fold but sense a large variety of bio-active small molecules. We first screened a number of these natural TetR proteins and identified one TetR family repressor that showed promiscuous sensitivity to melatonin in millimolar (mM) concentrations. Using directed evolution, we improved the repressor's sensitivity and specificity to sense melatonin in micromolar (μM) concentrations, which is within the physiological levels in mammalian pineal glands. In each round of evolution, libraries comprising 1000 – 20,000 variants generated with error-prone PCR or site saturation mutagenesis were transformed into *E.coli* and screened with a high-throughput fluorescence-based assay. Hits with greatest improvement in both sensitivity and specificity were selected, validated, and further evolved. In addition to melatonin-responsive repressors, we also identified NAS-responsive repressors, that could enable multiplexed monitoring of both melatonin and NAS. Interestingly, some repressor variants show reversed ON-OFF activity, which leads to the potential of engineering reverse melatonin-controlled transactivators. Both the repressors and the directed evolution method presented here provide a promising platform for development of genetically encoded neurotransmitter sensors and targeted expression of other neural modulators.