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Title: Genetically Encoded Biosensors of Nicotine and Opioids

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Abstract: We report the first-in-class biosensors of neural drugs spanning nicotine, fentanyl, methadone, and other clinically used and abused opioids. A choline-binding protein, OpuBC, was cloned to flank a circularly permuted GFP. The resulting scaffold was optimized to detect nicotine. Remarkably, the nicotinic biosensors also displayed low to moderate sensitivity to opioid drugs in a library screen. We performed directed evolution to generate iOpioidSnFRs with the desired sensitivity and selectivity. We demonstrate their application across quantitative biofluid measurements, live cell imaging, and in vivo photometry. Now, we are developing a continuous monitoring routine to find correlations in animal behavior and the drug waveform.