High Throughput Evolution of Near-Infrared Serotonin Nanosensors

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Release and reuptake of neuromodulator serotonin is central to mood regulation and neuropsychiatric disorders, whereby imaging serotonin is of fundamental importance to study the serotonin signaling system. Herein, we present a reversible near-infrared optical probe for serotonin that reports physiologically-relevant serotonin concentrations on relevant spatiotemporal scales, and is compatible with pharmacological tests. The probe responds with $\Delta F/F_0$ of up to 194% in the near-infrared fluorescence emission window of 1000-1300 nm, and is constructed from semiconducting single-walled carbon nanotubes (SWNT), which have shown utility for non-invasive through-skull imaging in rodents. Synthetic molecular recognition was utilized based on electrostatic pinning of bio-mimetic polymers, such as single strand DNAs (ssDNAs), to the surface of SWNT, whereby the polymer serves as the molecular recognition element, and the SWNT provides a near-infrared fluorescent signal in response to analyte binding. We develop a high-throughput screening platform for evolution of molecular selectivity for neuromodulator serotonin, in which systematic evolution of ligands by exponential enrichment is implemented on carbon nanotube surfaces, a process we've termed SELEC. 10^{11} unique SWNT-pinned ssDNA can be screened for their ability to bind a target analyte and provide a selective near-infrared fluorescence signal. Iterative selection of analyte-binding polymers that form a SWNT-surface-adsorbed phase for target recognition are identified through ionic desorption of sub-optimal polymers, and exponential amplification of nucleotides that recognize the target analyte. The best-responding serotonin nanosensor is shown to bind serotonin with a K_d of 6.3 μM, is shown to be reversible, and exhibits unaltered performance in artificial cerebrospinal fluid. Of importance to understanding pharmacology in the context of serotonin signaling, we show that out serotonin nanosensor does not respond to serotonin metabolites of 5-hydroxyindoleacetic acid (HIAA), 5-hydroxytryptophan (HTP), and 5-methoxytryptophan (MTP) and 5-HT receptor-targetting drugs. Lastly, nIRHT can be introduced into the brain extracellular space in acute slice, and can be used to image exogenous serotonin reversibly. Our results suggest evolution of nanosensors could be generically implemented to rapidly develop other neuromodulator probes, and that these probes can image neuromodulator dynamics at spatiotemporal scales compatible with endogenous neuromodulation. While we've implemented SELEC to develop an optical probe for serotonin herein, the platform is fundamentally generic for generating other optical probes of neurological relevance.