Tools of the trade

https://doi.org/10.1038/s41583-025-00972-9

A far-red dopamine sensor unlocks multiplex views of in vivo neuromodulation

The neurotransmitter dopamine (DA) has a central role in regulating diverse physiological functions, and its dysregulation has been implicated in numerous brain disorders. Yet DA does not act in isolation — its effects are shaped by its interactions with other neurotransmitters and downstream signalling pathways. Unravelling these intricate networks is essential for a comprehensive understanding of DA's functions.

Recent advances in genetically encoded neurotransmitter sensors, such as G-protein-coupled receptor activation-based (GRAB) sensors and dLight sensors, have enabled high-resolution in vivo imaging of DA dynamics in animal models. However, the fluorescent signals generated by these sensors have largely been limited to the green and red spectral ranges, constraining the simultaneous monitoring of multiple neurotransmitters and neurochemicals in complex neural networks. Overcoming this spectral bottleneck by pushing sensors into the far-red and near-infrared (NIR) region of the electromagnetic spectrum has proven challenging, because fluorescent proteins in the far-red or NIR range generally suffer from low brightness.

To address this problem, we adapted the established GRAB sensor design to use a circularly permuted HaloTag (cpHaloTag) domain as the fluorescent output module, generating a chemigenetic DA sensor named $GRAB_{HaloDA1.0}$ (HaloDA1.0). The cpHaloTag domain can be covalently labelled by various halogenated small-molecule fluorophores, offering high brightness and a choice of fluorescence wavelengths. When DA binds to HaloDA1.0, the receptor module undergoes a conformational change that is transduced to the fused cpHaloTag, altering the microenvironment of the covalently attached dye and producing a fluorescence change.

We showed that HaloDA1.0 exhibits good sensitivity, sub-second kinetics, efficient membrane trafficking, high molecular selectivity and minimal downstream signalling — comparable to fluorescent protein-based DA sensors. Furthermore, when labelled with far-red dyes, such as JF646 or SiR650, HaloDA1.0 showed minimal spectral overlap with existing green and red sensors, enabling simultaneous imaging of multiple neurochemical signals. For example, three-colour imaging in acute mouse brain slices revealed distinct release kinetics of DA, acetylcholine (ACh) and endocannabinoids during electrical stimulation, probably reflecting their different release mechanisms.

To extend this chemigenetic tool to in vivo applications, we conducted a systematic screen for dyes that balance sensitivity with the ability to penetrate the blood–brain barrier. Combining the optimal dye with a three-colour fibre photometry system, we simultaneously monitored DA, ACh and intracellular cAMP in medium spiny neurons expressing dopamine receptor-1 within the mouse nucleus accumbens, uncovering distinct dynamic patterns during various behaviors. These results suggest coordinated regulation of cAMP by DA and ACh under physiological conditions, a balance that we showed was disrupted by cocaine administration. We further demonstrated the multiplex imaging capability of HaloDA1.0 across various biological systems, including cultured neurons, acute brain slices, zebrafish and freely moving mice.

Studying multiple neurotransmitters in the same brain region has typically required separate measurements across different animals, with data aligned to external cues for comparison. By contrast, HaloDA1.0 enabled direct, simultaneous monitoring of multiple neurochemical dynamics, which is especially important for spontaneous, non-event-locked activity when no external cue is available for alignment-based analysis. We envision that the development and application of HaloDA1.0 will inspire similar strategies for expanding the sensor palette, adapting this chemigenetic strategy to other sensing modules and protein tags.

Despite its promise, HaloDA1.0 has limitations. Unlike conventional genetically encoded sensors, it requires exogenous dye labelling. Furthermore, its in vivo performance could be improved by optimizing its labeling efficiency. Nonetheless, with continued collaboration between biologists and chemists, we anticipate that such tools will be refined and expanded, accelerating the investigation of complex neurochemical networks and deepening our understanding of how physiological functions are orchestrated and regulated.

Yu Zheng
Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China.
e-mail: yu_zheng@pku.edu.cn

Competing interests
The author declares no competing interests.

Original article: Zheng, Y. et al. In vivo multiplex imaging of dynamic neurochemical networks with designed far-red dopamine sensors. *Science* **388**, eadt7705 (2025)