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Optical Probes for Neurobiological Sensing and Imaging

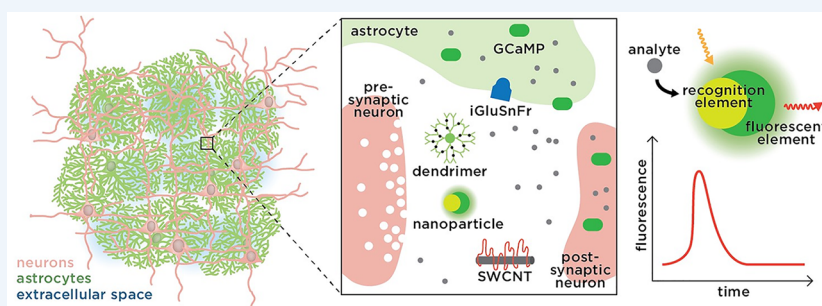
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S Supporting Information



CONSPECTUS: Fluorescent nanosensors and molecular probes are next-generation tools for imaging chemical signaling inside and between cells. Electrophysiology has long been considered the gold standard in elucidating neural dynamics with high temporal resolution and precision, particularly on the single-cell level. However, electrode-based techniques face challenges in illuminating the specific chemicals involved in neural cell activation with adequate spatial information. Measuring chemical dynamics is of fundamental importance to better understand synergistic interactions between neurons as well as interactions between neurons and non-neuronal cells. Over the past decade, significant technological advances in optical probes and imaging methods have enabled entirely new possibilities for studying neural cells and circuits at the chemical level. These optical imaging modalities have shown promise for combining chemical, temporal, and spatial information. This potential makes them ideal candidates to unravel the complex neural interactions at multiple scales in the brain, which could be complemented by traditional electrophysiological methods to obtain a full spatiotemporal picture of neurochemical dynamics. Despite the potential, only a handful of probe candidates have been utilized to provide detailed chemical information in the brain. To date, most live imaging and chemical mapping studies rely on fluorescent molecular indicators to report intracellular calcium (Ca^{2+}) dynamics, which correlates with neuronal activity. Methodological advances for monitoring a full array of chemicals in the brain with improved spatial, temporal, and chemical resolution will thus enable mapping of neurochemical circuits with finer precision.

On the basis of numerous studies in this exciting field, we review the current efforts to develop and apply a palette of optical probes and nanosensors for chemical sensing in the brain. There is a strong impetus to further develop technologies capable of probing entire neurobiological units with high spatiotemporal resolution. Thus, we introduce selected applications for ion and neurotransmitter detection to investigate both neurons and non-neuronal brain cells. We focus on families of optical probes because of their ability to sense a wide array of molecules and convey spatial information with minimal damage to tissue. We start with a discussion of currently available molecular probes, highlight recent advances in genetically modified fluorescent probes for ions and small molecules, and end with the latest research in nanosensors for biological imaging. Customizable, nanoscale optical sensors that accurately and dynamically monitor the local environment with high spatiotemporal resolution could lead to not only new insights into the function of all cell types but also a broader understanding of how diverse neural signaling systems act in conjunction with neighboring cells in a spatially relevant manner.

1. INTRODUCTION

In the past few years, consolidated efforts to understand neural circuitry have been driving technological innovation at many levels. These efforts include connectomics approaches to link fine-scale anatomical structures to neural function and functional recording approaches—optical, electrical, and both

simultaneously—to measure the activity of large populations of cells. However, efforts to map detailed chemical dynamics in the brain have not proceeded at the same pace as these other

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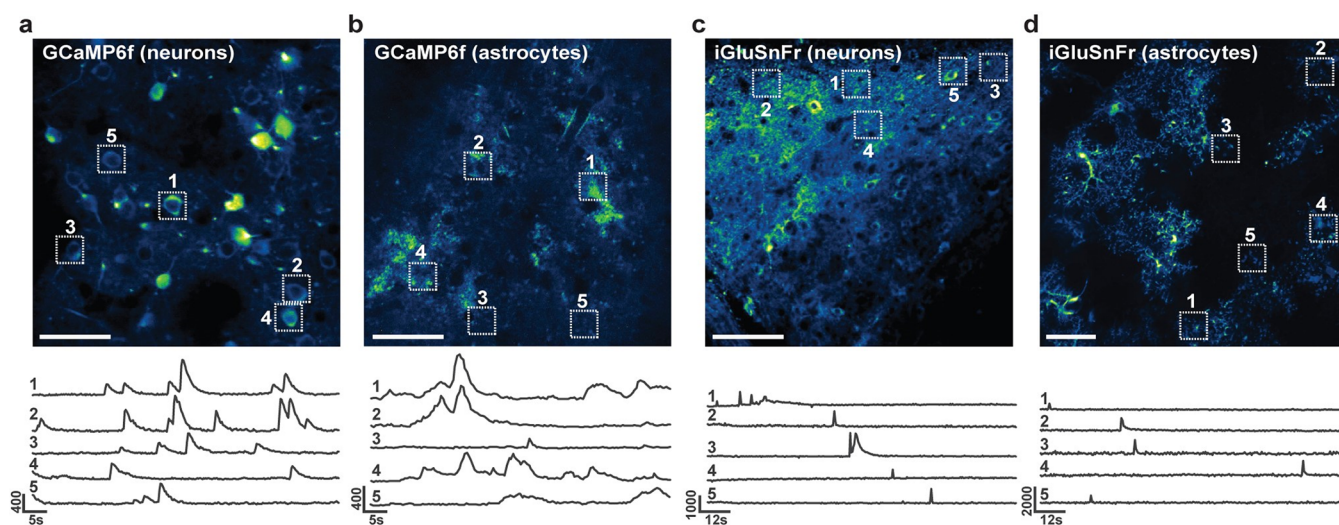


Figure 1. Two-photon imaging of cortical neurons or astrocytes expressing the genetically encoded indicators GCaMP6f and iGluSnFr in head-fixed mice (a, b) and slice (c, d) preparations. (a, b) Images of neuron- or astrocyte-specific expression of GCaMP6f and representative regions of interest (ROIs) (top) and corresponding average fluorescence intensity traces (bottom). (c, d) Images of neuron- or astrocyte-specific expression of iGluSnFr and representative ROIs (top) and corresponding average fluorescence intensity traces of sampled ROIs (bottom). Scale bars: (a, b) 40 μm ; (c) 80 μm ; (d) 35 μm . All fluorescence intensity traces in arbitrary fluorescence units. Data courtesy of Michael Reitman, Trisha Vaidyanathan, and Kira Poskanzer.

initiatives. Chemical signaling is one key to understanding brain function, since neural cells respond differentially to a diverse array of extracellular chemical stimuli with cascades of events involving defined classes of molecules. Thus, understanding chemical signaling in the brain is another critical component of these ongoing initiatives, requiring development of techniques that enable detection and measurement of chemicals in intact brain tissue.

In this Account, we describe recent efforts in the field of optical sensors and probes for chemical sensing in the brain. This class of imaging agents has key advantages for *in vivo* imaging, including the potential to obtain specific chemical information with spatiotemporal precision. Optical sensors for neurobiology require stringent parameters: they need to be sufficiently selective and sensitive to detect substances in the physiological range, exhibit reversibility for many *in vivo* applications, and have fast kinetics to closely match that of most neural activity. The last of these is especially critical for tracking neurochemical release and clearance during synaptic neurotransmission, which occur on the millisecond time scale.¹

We focus here on concepts that provide—or could potentially provide—the spatiotemporal resolution necessary to image chemical changes in neural circuits. We first introduce different classes of sensors and then present selected applications for the imaging of physiological ions and neurotransmitters in neural cells. To emphasize the complexity of sensing in the nervous system and assess the fact that most chemical probe development solely considers neuronal activity, we discuss non-neuronal cells, in particular astrocytes, as critical components of brain function. The sensing platforms in the following sections include (1) fluorescent molecular indicators, (2) engineered fluorescent proteins, and (3) nanoscale sensors. Depending on the neurobiological question at hand, intra- and/or extracellular sensing of molecules is critical. While electrophysiological measures can relay both types of information, they often lack robust spatial resolution, being limited to single sampling points or summative recordings.² Nanosensors and fluorescence-based techniques have the

capacity to overcome these barriers and enable measurements of robust spatiotemporal chemical information in both the intra- and extracellular environments.³ Thus, we discuss opportunities for which chemical sensors and probes may impart critical information to untangle intra- and intercellular communication among all neural cell types.

2. NEUROBIOLOGICAL NEED FOR CHEMICAL SENSORS

2.1. Neurobiological Need for Chemical Imaging Probes

To study the timing of neural events, neuroscientists measure the fast electrophysiological changes in brain cells. Amperometry⁴ and voltammetry⁵ are used to measure fast temporal neurochemical dynamics, while microdialysis⁶ is used to measure slower neurochemical dynamics. Although these techniques excel in temporal or chemical information, they lack the global spatial information of imaging methods. Additionally, many dynamic methods are neuron-centric by design and therefore do not account for the physiology of a variety of non-neuronal cell types. However, recent advances have demonstrated the important roles that non-neuronal cells—predominantly astrocytes—play in neural function to rapidly modulate chemical signals and ion concentrations.^{7,8} Thus, new techniques to map entire neurobiological circuits are needed in order to reveal relationships among neurophysiological events.

2.2. Functions of All Cell Types in Circuit

Signaling in neural circuits requires not only neuronal activity but also tight regulation by non-neuronal cells, particularly the largest class of non-neuronal (or glia) cells: astrocytes. For example, the major neurotransmitter in the brain, glutamate, is released during synaptic transmission and subsequently taken up by nearby astrocytic branches that can ensheath whole synapses.⁹ Rapid glutamate clearance (<10 ms) by astrocytes is integral to glutamatergic synaptic transmission. Improper clearance can lead to spillover, causing neuronal hyperexcitability and neurotoxicity.¹⁰ Extracellular potassium buffer-

Table 1. Optical Probes for Neurobiological Sensing Described in This Account

Substrate	Nanosensor	Probe Type	Size (nm)	Maximum Fluorescence (ΔF/F)	Sensitivity (Kd)/ Detection Limit ^b	Temporal Window /Decay-Rates	Application Range	Ref. ⁸
Calcium	OGB-1	Molecular Probe (MP)	1.5	+14	170 nM	<300 ms	<i>in vitro</i> (VT), <i>ex vivo</i> rodent	22,SR1
	Fura-2	MP			145 nM	100 s ^{-1,d}	(EX), <i>in vivo</i> mouse(IVM)	22,SR2
	Indo-1	MP		0.8 ^a	230 nM	130 s ^{-1,d}	VT, EX, IVM	22,SR3
	Fluo-3	MP		+40-200	345 nM	370 s ^{-1,d}	VT, EX, IVM	22,SR2
	ACR-1	MP		+6	0.49 μM	VT	VT, EX, IVM	27
	Cal-590	MP			561 nM	2 ms (rise-time)	VT, EX, IVM	28,29,30
	CaRubies	MP		+50	3-20 μM	3.6 ns ^e	VT, EX, IVM	31
	CaRuby-Nano	MP		+50	295 nM	0.55 ms (rise-time)	VT, EX, IVM	32
	GCaMP3	Genetically Encoded (GE)		+12	540 nM	650 ms ^f	VT, EX, IVM	35,36
	GCaMP6s	GE		+63	140 nM	550 ms ^f	VT, EX, IVM	35,36
	GCaMP6f	GE		+52	380 nM	140 ms ^f	VT, EX, IVM	35,36
	jRGECO1a	GE		+11	150 nM	200 ms ^f	VT, EX, IVM	37
	K-GECO1	GE		+12	315 nM	1.5 s ^f	VT, EX, IVM, zebrafish	38
	OpCaNS	Nanoparticle (NP)		2.5 ^a	110 nM ^c	<150 ms	VT	48
Sodium	SBFI	MP	120	+0.9	25 mM		VT, EX	23
	Sodium Green	MP			20 mM		VT, EX	24
	CoroNa Green	MP			82 mM		VT, EX	24
	ANG-1/2	MP		+1.3	38.7 mM	0.3 ns ^e	VT, EX	25,SR4
Potassium	Polymeric Na+ Nanosensor	NP	77		370 μM ^b		VT	47,49
	PBFI	MP			8 mM	0.3 ns ^e	VT	23,SR5
	APG-1	MP		+4.5	54 mM	t ₅₀ = 26.8 s	VT	25,SR6
	QDot K+ Sensor	NP		1.2 ^a	24 mM ^c		VT	47,51
Chloride	MQAE	MP			40 mM	<1 ms	VT, EX, IVM	26
	SuperClomeleon	GE			10 mM		VT	43
	mCl-YFP	GE		-0.45	14 mM		VT	44
	Clensor	DNA/Dendrimer		-0.45	5 mM ^b		VT	61
Glutamate	FLIPE	GE	120	+0.4	0.6 μM		VT, EX, IVM	41
	iGluSnFr	GE		+1.0	4.9 μM	4.3 ms ^e	VT, EX, IVM	42,SR7
	CPNP	NP		+9	3.8 nM ^b		VT, <i>in vivo</i> (zebrafish)	54
Dopamine	QuID	DNA/Dendrimer	100	+0.33	110 μM ^b			58
	Carbon-dots	Carbon-Based (CB)	5	58% (QY)	47 pM ^b	~100 s	VT	62,63,64
	SWCNTs	CB	0.4 - 2	+0.80	11 nM ^b	100 ms	VT	65,66,67
Zinc	UCNPs	NP	16		0.78 μM ^b	5 s	VT, EX, <i>in vivo</i> (zebrafish)	55
Acetylcholine	DNA-Dendrimer	DNA/Dendrimer	100	-6	200 μM ^b	~10 s	VT, EX	59
pH	I-Switch	DNA/Dendrimer		+5.0 ^a			<i>in vivo</i> (C. elegans)	60

^aFluorescence intensity ratio at two emission wavelengths. ^{b,c}Values are dissociation constants (K_d), unless otherwise noted: ^b indicates lower detection limit and ^c indicates half-maximal effective concentration of the sensor. ^dRate constant for the calcium unbinding reaction (k_{off}). ^eFast decay time component (τ). ^fHalf-decay rate. ⁸References for time characteristics of some indicators are listed as Supplementary References (SRs) in the Supporting Information.

ing by astrocytes performs a similar role, maintaining the proper extracellular environment suitable for neuronal action potential propagation.¹¹ Much work over the past decade has demonstrated that astrocytes also carry out active neural circuit regulation, including synaptic strength modulation¹² and lactate metabolism.¹³ One controversy in the field is whether astrocytes actively contribute to synaptic transmission by releasing their own neurotransmitters via a process called gliotransmission.¹⁴ Studies supporting gliotransmission suggest that astrocytes tune neuronal transmission by releasing glutamate,¹⁵ D-serine,¹⁶ or ATP¹⁷ directly into the synapse, altering current kinetics,¹⁸ modulating long-term potentiation,¹⁹ and coordinating synaptic networks.¹⁷ However, many argue that these results may be due to technical artifacts, and gliotransmission has not been directly observed. Clarifying whether and when astrocytes are capable of releasing neurotransmitters is thus crucial to our fundamental understanding of neurobiological circuits.

Questions about the functions of non-neuronal cell types in neural circuits extend beyond gliotransmission. Much recent astrocyte research involves teasing apart astrocytes' roles in regulating neuronal activity^{7,13,20} and as a separate system of information processing via analysis of spatiotemporally rich astrocytic intracellular Ca^{2+} transients (Figure 1). Considering astrocytes' direct role in maintaining the extracellular milieu, parsing the diverse signaling patterns observed intracellularly while simultaneously sensing extracellular shifts with high resolution is an attractive possibility. Chemical nanosensors are poised to illuminate some of these questions. Their ability to infiltrate compact extracellular spaces and report rapidly fluctuating molecular concentrations in a minimally invasive manner may also be used in conjunction with genetically encoded optical indicators to image intracellular and extracellular activity simultaneously. In all, novel methodologies to

visualize these heterocellular interactions using fluorescent or electrophysiological reporters paired with extracellular nanosensors could effectively probe a spatiotemporally rich environment and have implications spanning subcellular signaling to behavior.

3. PROBES FOR CHEMICAL MEASUREMENTS IN THE BRAIN

The successful development of an optical chemical probe is related to the nature of the physical platform on which it is based. This section presents a recent survey of different classes of probes (Table 1) that detect and measure ions and neurotransmitters with an emphasis on *in vitro* and *in vivo* neurobiological studies.

3.1. Molecular Probes

Since the advent of molecular probes in the 1980s,²¹ the number of indicators for fluorescent imaging of spatiotemporal ion dynamics has greatly expanded. These fluorophores are covalently coupled to a metal chelator, enabling fluorescence changes after analyte binding, thus providing a dynamic readout of local ion concentration. Oregon Green BAPTA (1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid, Fura-2, Indo-1, and Fluo-3 dyes, the homologues of the Ca^{2+} chelator EGTA (ethylene glycol-bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid),²² are the most broadly implemented dyes for reporting unbound Ca^{2+} . Detection of Na^+ and K^+ ions is commonly performed with SBFI (sodium-binding benzofuran isophthalate) and PBFI (potassium-binding benzofuran isophthalate), respectively. SBFI has been used to investigate Na^+ signaling in neurons and astrocytes.²³ Sodium Green and CoroNa Green are also commonly used dyes for Na^+ .²⁴ More recently, the family of Asante Natrium Green (ANG) and Asante Potassium Green (APG) has been developed with improved sensitivity.²⁵ The first indicators for Cl^- were based on quinolone: SPQ (6-

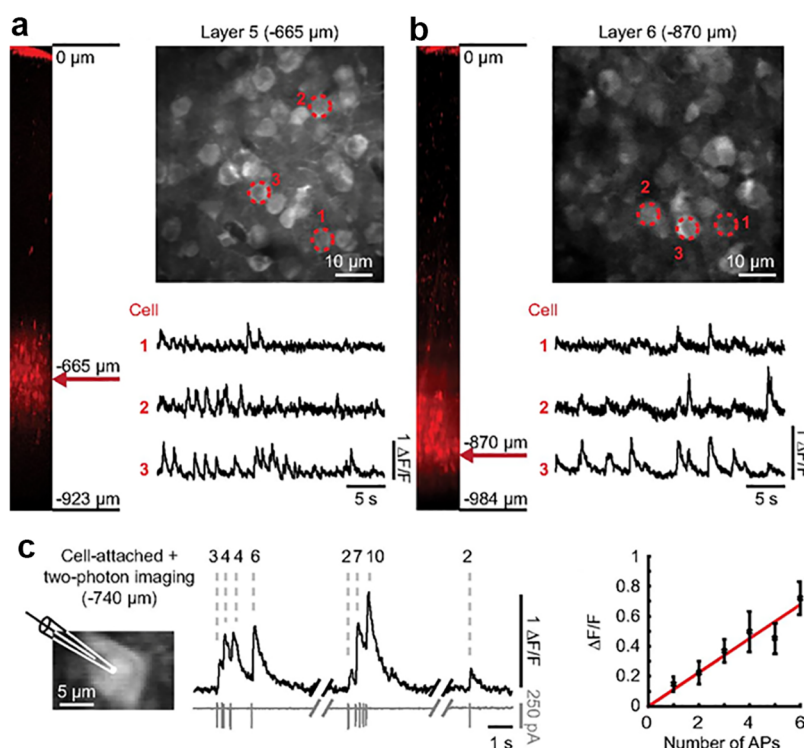


Figure 2. Two-photon Ca^{2+} imaging in deep layers of mouse visual cortex using Cal-590. The bottom traces in (a) and (b) indicate the fluorescence of spontaneous Ca^{2+} activity corresponding to the regions of interest circled in red. (c) Ca^{2+} transients corresponding to the number of evoked action potentials in layer 5 neurons. Reproduced from ref 30.

methoxy-*N*-(3-sulfopropyl)quinolinium), MQAE (*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide), and MEQ (6-methoxy-*N*-ethylquinolinium iodide). More recently, MQAE was imaged using two-photon (2P) excitation, an important step toward Cl^- imaging using molecular probes *in vivo*.²⁶

The main advantages of using chemical indicators include the ease of intracellular use and good quantum yield. However, each organic indicator may have different limitations, such as poor photostability, inadequate ion specificity, unwanted subcellular localization, or leakage from cells. In addition, they cannot easily be loaded intracellularly to specific genetically defined populations. Attempts at implementing these dyes for reliable extracellular sensing *in vivo* have proven subpar at best, as many diffuse quickly throughout the extracellular space, diluting their fluorescence readout. Ongoing efforts to improve the physicochemical characteristics of molecular ion indicators include optimizing their compatibility with modern confocal and multiphoton microscopy and developing probes with longer excitation wavelengths to reduce phototoxicity and photon scattering in tissue.

One newer indicator with a long excitation wavelengths is Asante Calcium Red-1 (ACR-1), which was created by conjugating BAPTA to seminaphthofluorescein and is used for live imaging in neuronal cultures.²⁷ Another Ca^{2+} dye, Cal-590,²⁸ has been used to record activity in isolated cortical neurons and *in vivo* neural populations at a depth of 900 μm into cortex (Figure 2).^{29,30} Other similar efforts include a family of red-shifted Ca^{2+} indicators, CaRuby³¹ and CaRuby-Nano,³² based on rhodamine-X. Overall, fluorescence methods based on organic dyes can provide real-time, *in situ*, and dynamic information. Dyes with longer wavelengths may meet the

criteria for precise spatiotemporal measurements in deep brain tissue.

3.2. Genetically Engineered Proteins

Genetically engineered proteins are minimally invasive, essential neuroscience tools for dynamic optical reporting of local analyte concentrations in specific cell types *in vivo*. While not the methodological focus of this Account, we would be remiss not to discuss their important role in chemical sensing due to their biologically advantageous design and widespread use. Here we touch upon some reporters while focusing on how their limitations illuminate technical gaps other probes may better address.

Genetically encoded fluorescent indicators are single fluorescent protein-based³³ or fluorescence resonance energy transfer (FRET)-based³⁴ systems expressed by cells of interest. Genetically encoded Ca^{2+} indicators (GECIs) are the most developed family of these indicators for neurobiological research. GECIs (e.g., those in the GCaMP family^{35,36} of indicators) exhibit high signal-to-noise ratios to report Ca^{2+} concentrations on the millisecond time scale. A variety of other Ca^{2+} indicators include red-shifted Ca^{2+} indicators such as jRGECO³⁷ and K-GECO,³⁸ voltage-sensitive indicators such as ArcLight³⁹ and ASAP1,⁴⁰ glutamate indicators such as FLIPE⁴¹ and iGluSnFr,⁴² and the Cl^- sensors SuperClomeleon⁴³ and mCl-YFP.⁴⁴ One advantage of these genetically encoded indicators is their ability to provide cell-type specificity. This has been useful in the expansion of Ca^{2+} imaging studies investigating the activity of neuronal subpopulations⁴⁵ and spatiotemporally rich astrocytic Ca^{2+} transients.^{7,46} Furthermore, the growing use of extracellular-facing indicators, notably iGluSnFr,^{7,46} has been a crucial step toward exploring spatiotemporal dynamics of the extracellular milieu.

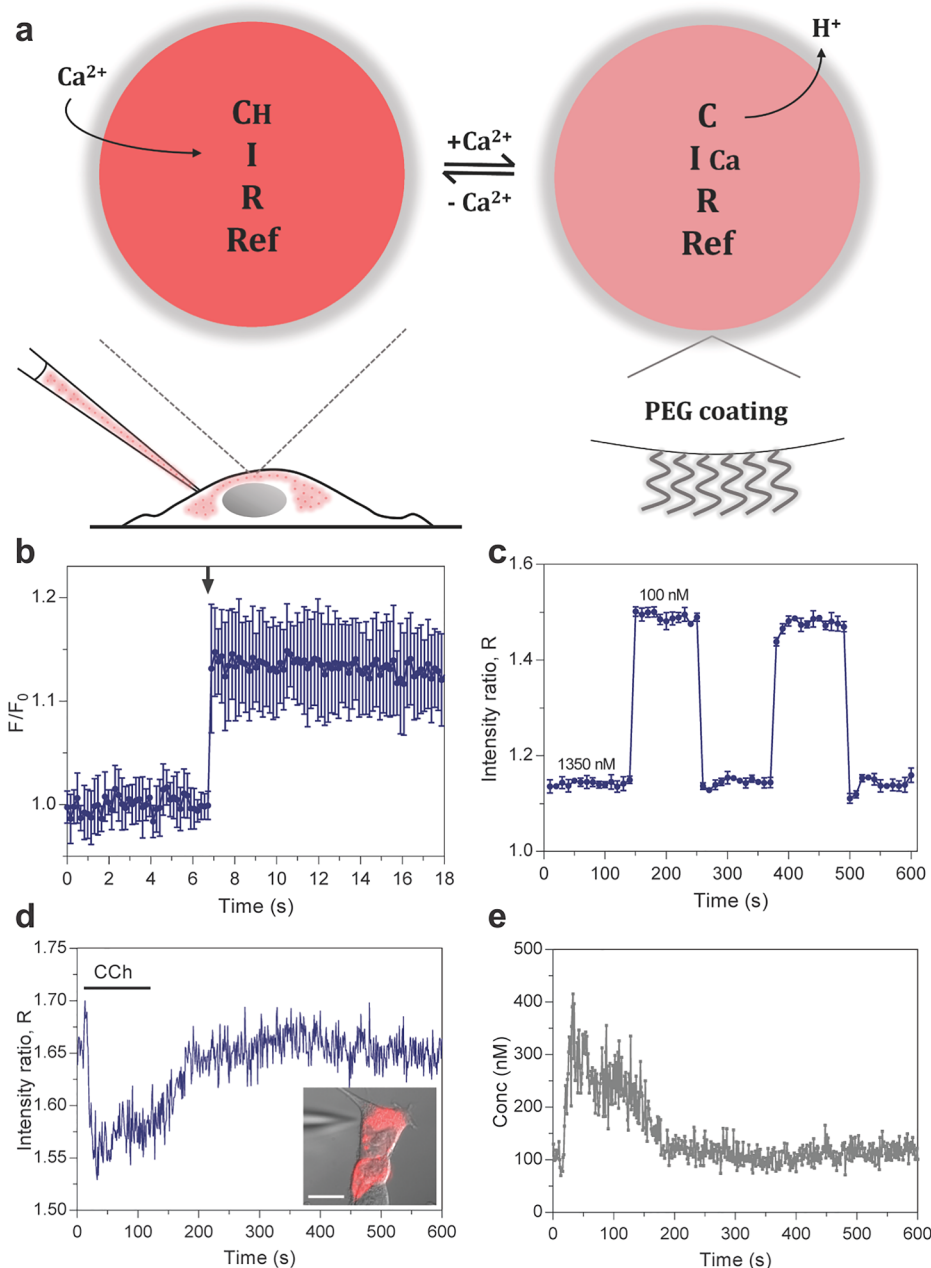


Figure 3. (a) Schematic of the OpCaNS Ca^{2+} nanosensor depicting four interchangeable components: chromoionophore (C), ionophore (I), ion exchanger (R), and reference dye (ref). (b) Instantaneous fluorescence response to two-photon Ca^{2+} uncaging of DMNP. (c) Reversible fluorescence response over repeated cycles by confocal microscopy. (d) Time-lapse confocal imaging of carbachol-induced Ca^{2+} release in nanosensor loaded HeLa cells (inset). (e) Fluorescence levels indicating that cytosolic elevations reach up to ~ 400 nM Ca^{2+} . Reproduced from ref 48. Copyright 2017 Springer Nature.

Although developments increasing signal-to-noise ratios and red-shifting excitation spectra have improved these tools, the application of genetically encoded indicators can still pose limitations. Engineered protein expression itself may alter normal neurophysiology. Genetic expression may occur over long development timelines (often months), and the indicators may differ in their reporting dynamics over the lifetime of their expression. Additionally, these indicators are subject to nonspecific expression patterns depending on the genetic system used to drive expression. Optimization on a case-by-case basis is required.

Currently, most genetically encoded indicators have been developed for intracellular measurements, leaving extracellular

sensing of ions and neurotransmitters relatively undeveloped. The need for broader sensing capability is of the utmost importance, but the development pipeline for genetically encoded probes can be long. Thus, the remainder of this Account focuses on alternate, nongenetically encoded sensing techniques that may address some of these limitations through rapid, flexible probe development, targeted diffusion through the extracellular space for unbiased reporting of extracellular chemicals, and optical compatibility with genetically encoded indicators for simultaneous measurements.

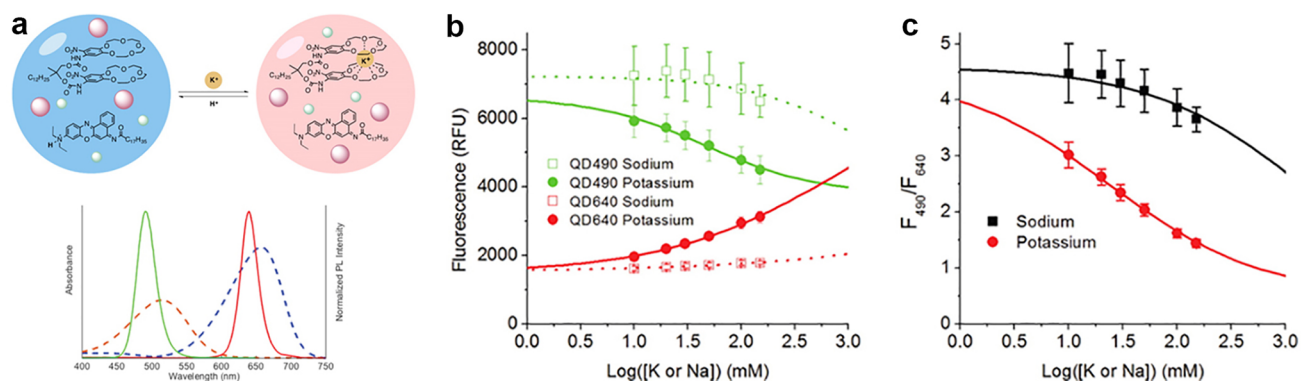


Figure 4. QD-based K^+ -selective nanosensor. (a) Schematic of the nanosensor mechanism. The selective recruitment of K^+ into the nanosensor core produces changes in the absorbance spectrum of the quencher (chromoionophore), which are detected by two QDs via the FRET mechanism. (b, c) Sensor calibration curves of (b) individual QDs and (c) their ratio in response to sodium and potassium. Reproduced from ref 51. Copyright 2016 American Chemical Society.

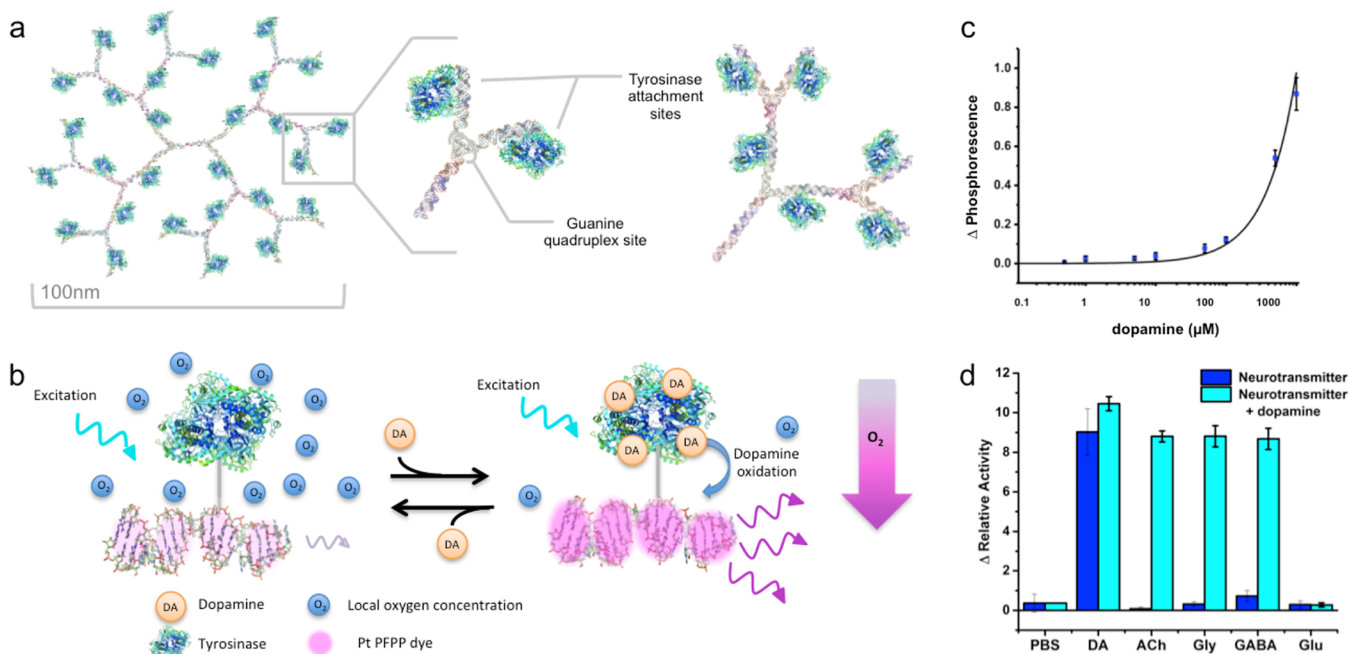


Figure 5. DNA nanoprobe for dopamine sensing. (a) Schematic of the DNA nanostructure conjugated with tyrosinase. (b) Schematic of the mechanism of dopamine detection. (c) Sensitivity and (d) selectivity profiles of the sensor. Reproduced from ref 58.

3.3. Nanoscale Sensors

Nanoparticle (NP)-based sensing platforms provide advantages due to their modular design, which enables the exchange of components to tune the response for dynamic range, selectivity, and a range of wavelengths.⁴⁷ For example, NPs have been utilized to investigate intracellular Ca^{2+} concentrations (Figure 3),⁴⁸ intracellular Na^+ dynamics,⁴⁹ extracellular K^+ dynamics^{50,51} and for *in vivo* biosensing.^{47,52} They have also shown sufficient selectivity against major interfering ions at their respective physiological ranges.^{48,50} The following sections focus on applications of fluorescent nanoscale probes of different compositions for chemical measurements in neural cells.

3.3.1. Nanoparticles. Nonorganic materials, such as semiconductor quantum dots (QDs), are intrinsically fluorescent and provide superior optical properties. The exceptional photostability of QDs relative to organic fluorescent dyes as well as the tunable emission, broad absorption spectrum, high quantum yield, and high molar extinction coefficients of QDs

make them particularly suitable for repeated imaging.⁵³ However, they can present biocompatibility and cytotoxicity problems due to size, surface charge, coating materials, or dosage and duration of exposure.

A FRET-based NP sensor⁵¹ capable of detecting real-time K^+ concentrations using QDs has been reported (Figure 4). In this sensor design, the NP contained nonfluorescent K^+ -selective ionophores in combination with pH-sensitive chromoionophores and inert QDs. The sensing mechanism was based on ion extraction of K^+ by the ionophore with concomitant deprotonation of the chromoionophore to preserve electro-neutrality. Optically, the chromoionophores acted as quenchers to modulate the emission of the QDs, producing a ratiometric signal dependent on the K^+ concentration. Importantly, the time-resolved fluorescence measurements were K^+ -dependent, indicating that the sensors could be used for fluorescence lifetime imaging microscopy (FLIM).

Conjugated polymer NPs (CPNPs) are a more biocompatible option than QDs, with superior photostability compared

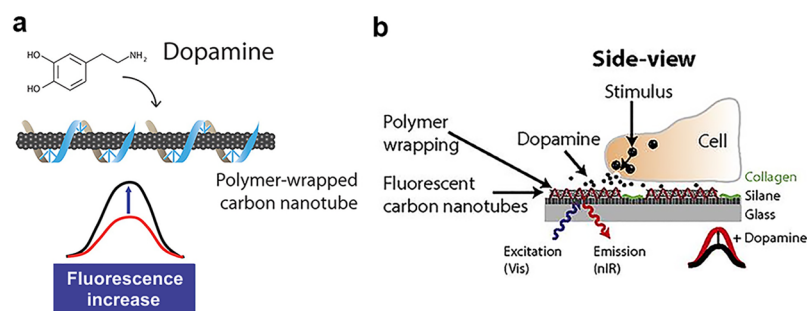


Figure 6. Fluorescent SWCNT nanoprobe. (a) Schematic of dopamine detection. Adapted from ref 66. Copyright 2013 American Chemical Society. (b) Detection of dopamine release by PC12 cells using a carbon nanutube array. Adapted from ref 67.

with molecular indicators. For example, fluorescent CPNPs conjugated to phenylboronic acid (PBA) were used for dopamine detection. When PBA on the surface is bound to dopamine, a photoinduced charge transfer occurs between the complex and the CPNP core. Injection of CPNPs into the brain ventricle for rapid dopamine detection in zebrafish larvae brains demonstrated the utility of CPNPs for *in vivo* imaging.⁵⁴

Photonic upconversion nanoparticles (UCNPs), typically lanthanide-doped, have also been used as a sensor platform. Upconversion luminescence (UCL) is produced when absorption of photons results in the emission of photons of a shorter wavelength. In one study, a Zn^{2+} fluorescence-based UCNP probe was introduced;⁵⁵ in the absence of Zn^{2+} , UCL was quenched by chromophores on the surface of the NPs. The presence of Zn^{2+} inhibited the FRET and recovered the UCL, enabling detection of Zn^{2+} . *In vitro* and *in vivo* detection of Zn^{2+} was demonstrated in mouse brain slices and zebrafish, respectively.

3.3.2. DNA and Dendrimers. Nucleic acids are a versatile material for developing novel sensing platforms. Structural DNA nanotechnology uses short oligonucleotides that form into self-assembled arrangements and complex structures.^{56,57} Recently, these DNA-based nanostructures have been exploited to detect neurotransmitters and ions.

First, a quadruplex integrated DNA (QuID) nanosensor platform immobilizes enzymes as recognition elements for selective detection of dopamine⁵⁸ (Figure 5). The DNA quadruplex architecture was designed to work in physically constrained environments (~ 100 nm) with high structural flexibility, homogeneous size, and fully reversible signal. The mechanism used tyrosinase to degrade dopamine, consuming oxygen and attenuating an oxygen-responsive phosphorescent indicator. Second, acetylcholine-selective nanoprobe based on a DNA dendrimer scaffold have also been designed, incorporating cholinesterase and a fluorescent pH indicator.⁵⁹ These probes were highly selective and reversibly responded to neurophysiological levels of acetylcholine. Additionally, application in neural tissue slices indicated that the sensors respond reversibly to changes in acetylcholine. These modular DNA-based probes are a powerful platform to facilitate the development of new sensing tools for neurobiology.

In a separate study, DNA-based nanoprobe termed I-switch⁶⁰ were utilized for detection of subcellular pH, with targeting to specific organelles. Also based on a pH mechanism, the ratiometric Cl^- sensor called Clensor⁶¹ was used to track lysosomal Cl^- concentrations in *Drosophila* cells.

3.3.3. Carbon-Based Nanomaterials. Carbon-based nanomaterials (CNMs) provide sensing platforms that are advantageous for sensitivity, selectivity, and tunability. CNMs

exhibit high resistance to photobleaching and low toxicity.⁶² Carbon dots are a class of quasi-spherical zero-dimensional carbon NPs.⁶³ In one approach, fluorescent sulfur-doped carbon dots were used to detect dopamine in PC12 cells.⁶⁴ Single-walled carbon nanotubes (SWCNTs) have recently drawn attention as biosensor scaffolds for neurotransmitter detection. SWCNTs show potential for *in vivo* optical biosensor design due to their intrinsic fluorescence in the near-infrared region (900–1400 nm) and optical customizability primarily dependent on the nanotube diameter and chiral angle.⁶⁵ DNA-wrapped SWCNTs responding to dopamine were developed to exhibit full reversibility and high selectivity compared with homologues.⁶⁶ In a recent study, SWCNT sensor arrays were used to map dopamine release sites in PC12 cells with high spatiotemporal resolution (Figure 6).⁶⁷ After potential biocompatibility issues are addressed, SWCNTs could clearly be extended into *in vivo* neurobiological imaging.

4. CONCLUSION AND PERSPECTIVE

Recent advances have extended the options for optical sensing in the brain. For molecular dyes, current efforts for improving physicochemical properties include enhanced sensitivity, brightness, and shifting of spectra to longer excitation and emission wavelengths. Fluorescent NPs offer new opportunities for intracellular and extracellular sensing. The examples reported in this Account demonstrate the potential benefits shown by the use of these probes in neural cells and circuits. We expect that many of the challenges identified in this Account will be overcome in the coming years and that new tools will enable neurobiologists to address fundamental questions about brain function and behavior.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.accounts.7b00564.

Supplementary references for Table 1 (PDF)

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Notes

The authors declare no competing financial interest.

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Guoxin Rong obtained his Ph.D. from Boston University and has worked as postdoctoral research associate in Heather Clark's lab at Northeastern University. His research interest focuses on utilizing nanosensors to illuminate neural dynamics.

Kira E. Poskanzer is an Assistant Professor in the Department of Biochemistry & Biophysics at UCSF. Her lab studies bidirectional signaling between cortical neurons and astrocytes using optical and electrophysiological techniques.

Heather A. Clark is a Professor with appointments in the Department of Bioengineering and the Department of Chemistry and Chemical Biology at Northeastern University. Her work focuses on nanosensor development and applications to biological imaging.

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