Genetically targeted chemical assembly

Anqi Zhang[1,2], Yuanwen Jiang[1], Kang Yong Loh[3], Zhenan Bao[1]*, and Karl Deisseroth[2,4,5]*

Affiliations:

[1] Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA

[2] Department of Bioengineering, Stanford University, Stanford, CA 94305, USA

[3] Department of Chemistry, Stanford Chemistry, Engineering & Medicine for Human Health (ChEM-H), Stanford University, Stanford, CA 94305, USA

[4] Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305, USA

[5] Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA

*Correspondence: deissero@stanford.edu (K.D.), zbao@stanford.edu (Z.B.)

Abstract

Cell-type-specific interfaces within living animals, suitable for communicating with identifiable cells over the long term, would be invaluable across many scientific and medical applications. However, biological tissues exhibit complex and dynamic organization properties which pose serious challenges for chronic cell-specific interfacing. A novel technology, combining chemistry and molecular biology, has emerged to address this challenge: genetically targeted chemical assembly (GTCA), in which cells are genetically programmed to chemically incorproate non-biological structures *in situ*. Here, we discuss recent progress in genetically-targeted construction of materials, and outline opportunities that may expand the GTCA toolbox: 1) possible specific chemical processes involving novel monomers, catalysts, and reaction regimes: both *de cellula* (from the cell) as previously described, and *ad cellula* (toward the cell); 2) diverse new GTCA-compatible reaction conditions, with a focus on light-based patterning; and 3) potential applications of GTCA for both research and clinical settings.

Introduction

The intricate and dynamic physical architectures of biological systems, particularly the nervous system, pose significant challenges in establishing cell-type-specific and non-invasive connections with external interfaces. The human brain, for example, contains billions of neurons, each using electrical information-propagation signals on the millisecond timescale. Relative to these natural properties, existing hardware for studying the brain still lack sufficient spatiotemporal resolution, sensitivity, specificity, and plasticity. Due to these mismatches with biological elements, and an inability to target specific cell types[1-10], modern devices cannot achieve the goal of high-content, specific, seamless, and non-invasive integration.

A fundamentally new approach to address this mismatch is to genetically program specific cells within living tissue (for instance, neurons in the brain), endowing the intrinsic ability to incorporate materials and build structures with desired forms and functions. It is possible to modify and regulate biopolymer synthesis from natural building blocks[11-13], but these approaches have primarily focused on microorganisms, and the diversity of natural substrates remains limited in comparison to non-natural building blocks that could theoretically be recruited for a vast new domain of materials synthesis and assembly within living systems. Previous works have shown that conductive polymers can be directly synthesized in living brains without compromising brain fucntion[14], although these approaches have not yet provided ability to target specific cell types.

We have taken the first step in genetically-targeted synthesis using non-biological reactions and reactants to establish the field of genetically targeted chemical assembly, or GTCA[15,16]. The first instantiation of GTCA used cell-specific genetic information to guide neurons to initiate deposition of polymer materials *in situ* with a variety of electrical conduction properties[15,16].

In this perspective, we lay out potential strategies to broaden the scope of GTCA and stipulate future therapeutic applications. First, we outline the broad potential of GTCA by highlighting both reported GTCA methods and new approaches we have established for chemical synthesis of materials in living systems (including an alternative *ad cellula* approach that allows *ex situ* attachment of pre-synthesized materials to cells). Second, we review potential diverse GTCA reaction conditions that may be imposed through modulation of light, pH, heat, and other signals. Third, we discuss potential applications of the broad GTCA concept in both neuroscience research and the treatment of disease, in both the central and peripheral nervous systems, noting existing challenges and future opportunities.

I. Genetically targeted chemical assembly of functional materials

The initial demonstration of GTCA used cell-specific genetic information to guide neurons to deposit conductive or insulating polymers *in situ*[15]. Specifically, neurons, including in non-transgenic mammals, were genetically engineered to express a peroxidase enzyme, which catalyzes hydrogen peroxide (H_2O_2)-enabled oxidative polymerization. We have demonstrated the in vitro and in vivo synthesis of both conductive polymers, such as polyaniline (PANI), and insulating polymers, such as poly(3,3'-diaminobenzidine) (PDAB). Electrophysiological and behavioral studies confirmed that deposited polymers modulated membrane capacitance cell type-specific behaviors in living neural systems.

Despite this initial success, this proof-of-concept system had a key limitation: the peroxidase was not specifically targeted in a robust manner to the plasma membrane. On the other hand, developing an efficient method to ensure complete localization of the reaction centers on the external side of the membrane was critical for this and other applications, because live cells are

not permeable to large precursors or materials, and localizing reactions to the extracellular space could limit adverse effects on native intracellular chemistry[17-19]. We recently introduced a second generation GTCA technique. This upgraded method allows for precise polymer assembly by incorporating horseradish peroxidase (HRP) in a highly localized manner on the plasma membrane of primary neurons, while minimizing its retention in the intracellular space[16] (**Fig. 1a**). Upon addition of polymer precursors and H_2O_2, the membrane-displayed HRPs facilitated oxidative polymerization on targeted neurons. The synthesized polymers formed dense clusters around the living neuron membrane of interest, and neurons remained viable after polymerization[16], establishing a robust foundation for future applications discussed in this perspective.

II. Diversifying the GTCA chemistry toolbox

To expand the capabilities of GTCA, a larger chemistry toolbox is in development. This section describes several novel and general approaches applicable to a variety of polymers and nanomaterials, selected for compatibility with modern genetic engineering technologies, suitability for wildtype (non-transgenic) animals, and tolerability (minimal toxicity) for living biological systems ultimately with complexity and fragileness as the mammalian brain.

a. Expanding the HRP/H_2O_2 system to modulate reaction rate and polymer conductivity

In our previous work[15,16], we discovered that polymer precursors (monomers or dimers) with lower oxidation potential are preferable for peroxidase-catalyzed oxidative polymerization that is compatible with a low concentration of H_2O_2 in physiological solutions. Another well-studied, biocompatible conductive polymer suitable for expanding the GTCA system is polypyrrole (PPy), which may have a better biocompatibility[20] than PANI, and offer fine-tuning of oxidation potential

and polymerization kinetics through side-chain functionalization[21] and copolymerize with other pyrrole derivatives (**Fig. 1b**). In electrochemical and chemical polymerization of pyrrole, the initial oxidation of pyrrole monomer to bipyrrole is the rate-limiting step, as the oxidation potential of pyrrole is much higher than that of bipyrrole and other oligomers[22,23]. Therefore, adding bipyrrole to the polymer precursors is expected to significantly increase the reaction rate.

Alternatively, another pyrrole derivative (3,4-ethylenedioxypyrrole or EDOP), where 3,4-alkylenedioxy substitution lowers the monomer oxidation potential and restricts polymerization to the 2- and 5- positions, can also be used to facilitate polymerization and reduce backbone imperfections[24,25]. Copolymerization of PPy with other conductive polymers, such as polythiophene[26] and poly(3,4-ethylenedioxythiophene) (PEDOT)[27] may lead to new materials with tunable intermediate properties.

In addition, to increase the conductivity of PPy synthesized under biocompatible conditions, doping agents can be used or added to the precursor solution and covalently incorporated into the polymer structures (**Fig. 1c**). For example, self-doping of PPy can be achieved by adding alkyl sulfonate sidechains on pyrrole precursors[28]. As the chain length of the conductive polymer increases, its solubility in water decreases significantly, which limits the conjugation length and leads to low polymer conductivity. Using sulfonate groups not only increases the doping level, but also increases the polymer solubility, which could further increase the conductivity.

In enzymatic synthesis of conductive polymers, another commonly used redox mediator and doping agent is 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)[29,30], an effective peroxidase substrate. ABTS can be oxidized to generate a radical cation that in turn chemically oxidizes pyrrole, and its sulfonate groups can also be electrostatically incorporated into the PPy backbone. Another approach is to use a sulfonate acid polymer, such as poly(2-acrylamido-2-

methyl-1-propanesulfonic acid) (PAAMPSA)[31,32] or sodium poly(styrene sulfonate) (PSS)[27], as both dopant and template for PPy synthesis, which can yield water-dispersible polymers with potential for significantly increased conjugation length and conductivity.

Notably, previous studies have demonstrated that incorporating a small percentage of polydopamine (PDA) into PPy can also increase polymer conductivity. This is because dopamine is negatively charged and can be potentially function as a dopant; the resulting π–π stacking between the PDA and PPy stabilizes charge carriers, and PDA may lead to better adhesion between PPys and maybe with tissue surface[33-35]. Together, this diverse set of PPy precursors and doping agents may provide new functionality by enabling fine-tuning of reaction rate and polymer conductivity, thereby enabling adjustable modulation of cellular membrane properties (for example in neurons, modulation of membrane capacitance and action potential firing).

b. Exploring other oxidases that do not require external H_2O_2

In the HRP/H_2O_2 GTCA system, reactions are carried out in biocompatible aqueous solutions with a low concentration of H_2O_2 (≤ 0.05 mM) to trigger a one-time oxidative polymerization reaction[16]. However, central to the purpose of GTCA is the requirement that synthesized polymers must remain within the intact living system for an extended period of time in order to achieve chronic modulation. As a result, potential oxidative toxicity of H_2O_2 in the long term motivates exploration for other possible enzymes that can catalyze the oxidative polymerization without need for external H_2O_2.

Within the oxidative enzyme family, besides the peroxidases, oxidases are another major group that can catalyze redox reactions by converting molecular oxygen (O_2) from air into reactive oxygen species (ROS). The first candidate is nicotinamide adenine dinucleotide phosphate

(NADPH) oxidase (NOX), the only mammalian enzyme dedicated to ROS generation[36]. The NOX enzymes are transmembrane proteins that transport an electron from cytosolic NADPH to O_2 on the extracellular side of the membrane to produce superoxide anion[36], where the unpaired electron imparts high reactivity. Certain isoforms of NOX, including NOX1, NOX2, and NOX4, can be upregulated in neurons[37]; previous studies in this setting have reported that NOX requires continuous metabolism of glucose to supply its NADPH substrate[38], and therefore in principle polymerization reactions can be controlled by glucose level, although presence of NOX and glucose at baseline in unmodified cells may pose challenges for ensuring specificity of genetic targeting. Relevant to this consideration, we have identified two other oxidase candidates (glucose oxidase[39] and laccase[40]) that are broadly distributed in fungi and plants but not mammals. Both oxidases are able to catalyze oxidative polymerization of PANI[41,42] and PPy[43,44], although their expression and function in mammalian systems need to be tested and optimized.

c. Genetically-enabled conjugation of pre-synthesized materials

In our initial work on GTCA, we focused on assembly of functional materials with construction starting from the cell membrane. Although many reactions could potentially fall within the scope of this from-the-cell or "*de cellula*" regime, it is important to acknowledge that only a subset of reactions can be practically performed, due to inherent limitations imposed by the underlying chemistry and biology. First, all the reactants and conditions must be biocompatible (with maintained robust cell structure, function, and overall health in tissue after the reaction). Second, the reaction type must be based on existing genetically-encodable enzyme capability, and only six groups of reactions can occur under enzymatic catalysis: 1) redox reactions by oxidoreductases, 2) transfer of a functional group (e.g. a methyl or phosphate group) by

transferases, 3) hydrolysis by hydrolases, 4) bond cleavages by lyases, 5) isomerization by isomerases, and 6) covalent linkages by ligases[45]. Due to these categorical and biocompatibility limitations, many reactions cannot occur under suitable conditions in the physiological environment. Here, we summarize two alternative approaches that explore the distinct toward-the-cell or "*ad cellula*" regime, which works around these limitations via *ex situ* attachment of pre-synthesized polymers or nanoparticles to living cells with cell-type specificity.

First, the biorthogonal chemistry toolbox[46] can be used with GTCA to selectively introduce abiotic functional groups. Four major biomolecules, namely, nucleic acids, proteins, carbohydrates, and lipids have been endowed with bio-orthogonal chemical moieties to be metabolized and incorporated into biological systems. Here we note a general enzyme-based activation strategy to unmask caged amino acids, monosaccharides, and lipids to be incorporated as azido or alkyne modified metabolites (**Fig. 2a**). Enzyme-substrate pairs orthogonal to native biochemical reactions could be utilized[47,48]. Hydrolases such as esterase enable the hydrolytic cleavage of ester groups could be utilized to caged acetylated azido-monosaccharides[46]. Specifically, N-azidoacetyl-mannosamine (ManNAz), a metabolic precursor modified with azide groups, can enter the sialic acid biosynthesis pathway and eventually be anchored on the cell membrane[49,50]. Once internalized, these unnatural sugars can be metabolized by native glycosyltransferases and incorporated into cell surface glycans to enable azide modification of the glycocalyx layer (including glycoproteins, and glycolipids) on membranes of targeted cell types. In addition, azido labelled extracellular membrane proteins could also be generated via genetic code expansion with non-canonical amino acids. Recent reports have incorporated artificial biosynthetic pathways to enable genetic targeting in this process[51-54]. Pre-synthesized polymers and nanoparticles functionalized with dibenzocyclooctyne (DBCO) groups can then be selectively anchored on the membrane through

the alkyne-azide cycloaddition reaction forming a stable triazole. One potential caveat about this approach is that the unmasked molecules or metabolites might diffuse into neighboring cells through gap junctions[55], resulting in decreased selectivity of surface labeling.

Delivery of functional groups to the membrane for GTCA could also be achieved using modular protein-peptide interaction systems (leveraging the molecular strategies for gene delivery we used for expressing membrane-displayed HRP in primary neurons[16]). One example that could be incorporated as a component of *ad cellula* GTCA would be the SpyTag/SpyCatcher system[56]; the SpyTag fragment is a small peptide (13 amino acid residues) that interacts with the SpyCatcher protein to form an isopeptide bond that is highly specific, modular, and stable in living cells. Through membrane expression of SpyCatcher, and linking the SpyTag peptide to pre-assembled or partially-assembled materials for GTCA synthesis such as polymers and nanoparticles, selective localization of the resulting designed structure only to cells with SpyCatcher on the surface could be achieved[57] (**Fig. 2b**). Alternatively, the SpyTag peptide could be fused to other membrane-expressed proteins, with the SpyCatcher conjugated onto the materials[58]. Orthogonal systems like SnoopTag/SnoopCatcher[59] can be combined with SpyTag/SpyCatcher to enable simultaneous targeting of two different cell types and materials. We have also demonstrated *ad cellula* GTCA with the streptavidin/biotin system, where the streptavidin was expressed on the membrane, to bind biotin-conjugated gold nanoparticles[16].

III. Exploring alternative GTCA strategies: genetically-targeted reaction conditions

In our original demonstration of GTCA, the delivery of peroxidase-encoding vectors and monomer solutions relied on injection and subsequent diffusion, resulting in limited spatial resolution. In addition, the HRP/H_2O_2 system was only capable of inducing a single reaction, rather

than temporal patterning. To better match the complexity and plasticity of biological assembly, here we introduce other reaction conditions, with a focus on light-based approaches.

Light-driven technologies not only enabled photolithography and 3D printing, but also fueled the rapid advancements in tools for biological systems, such as optogenetics[60-62]. We anticipate light-based GTCA could further expand the scope of GTCA. First, the light source may be easily focused to a diffraction limited spot (down to the submicron scale). In conjunction with a scanning system, the light spot may be aimed at any location of interest to form patterns with high spatial precision (to the extent compatible with light-scattering effects, which can be ameliorated with multiphoton methods[60-62]). Second, the intensity and duration of light may be tuned on-demand such that a reaction is well controlled with a high temporal resolution. Third, light may initiate a range of photochemical (radical chemistry, fluorescence) and photophysical (photovoltaic, photothermal) responses for a multitude of applications.

a. Mode 1: genetically targeted photosensitizers enabling 3D in vivo photolithography

The state-of-the-art 3D photolithography technique, two-photon polymerization, can create arbitrary 3D nano/micro-structures with sub-100 nm resolution (**Fig. 3a**). To write a shape, a femtosecond laser beam is tightly focused onto a photoresist block made of photoinitiators and monomers. Multiphoton absorption by photoinitiators occurs only where light intensity is the highest, which confines polymerization at the sub-100 nm focal spot; microstructures are created through laser scanning to form predetermined geometries. Here, we describe the concept of genetically targeted *in vivo* photolithography, to create arbitrary 3D conductive "neural lace" connecting brain cells and regions (**Fig. 3a**). In this system, genetically encoded photosensitizers[63] that produce reactive oxygen species (ROS) upon illumination are used as photoinitiators. They

function as membrane-displayed reaction centers, facilitating photopolymerization of conductive polymer precursors. The ROS produced by photosensitizers is only generated locally *in situ* at the illuminated area within a small distance from the cell membrane and is expected to be consumed instantaneously by the monomers, thus circumventing the toxicity of externally delivered H_2O_2. With digital micromirror device (DMD) or spatial light modulator (SLM) based one-photon or two-photon illumination[64], writing resolution may reach the single-cell, single-neurite, or even near single-synapse level.

Critical to the approach described here has been our initial systematic analysis of the genetically encoded photosensitizers which could be suitable for polymerization on living neurons; **Table 1** lists representative classes of genetically encoded photosensitizers that have been reported so far (although we first generated this list several years ago, the key players remain the same).

The first genetically-encoded ROS-generating protein reported was KillerRed[65], which produces ROS upon illumination with red light (excitation maximum of 585 nm) and has been commonly used for cell ablation[66]. However, a substantial disadvantage of KillerRed variants is that they cannot polymerize 3,3-diaminobenzidine (DAB) for electron microscopy applications[67], suggesting lower oxidation capability than HRP. In addition, efforts to develop genetically encoded photosensitizers have focused mini singlet oxygen generator (miniSOG)[68], a fluorescent flavoprotein that binds to flavin mononucleotide (FMN) an efficient photosensitizer with a relatively high quantum yield in producing singlet oxygen $^1O_2$. Notably, illumination of miniSOG generates sufficient $^1O_2$ to locally catalyze the polymerization of DAB for electron microscopy[66,69], and new mutants of miniSOG increase $^1O_2$ production by up to tenfold[67,70-72]. Lastly, a new type of genetically targeted fluorogen-activating protein FAPdL5** was recently developed to generate $^1O_2$ under near IR (NIR) illumination (669 nm)[73]. Tissue penetration ability of NIR lasers opens

up deep tissue applications, but it remains to be tested if the $^1O_2$ generated by FAPdL5** would be sufficient to trigger *in situ* polymerization; notably, FAPdL5** also requires incubation in the externally delivered cofactor iodine-substituted dye.

As all three photosensitizers have been expressed on cell membranes, we were able to evaluate ablation efficiency by comparing light doses required for cell ablation (**Table 1**). Note that in all reports on membrane-targeting photosensitizers, the photosensitizers were expressed on the inner leaflet of the membrane. In contrast, in our GTCA design[74], the photosensitizers are expressed on the extracellular side; therefore cell ablation dose should be significantly increased, and polymerization on the cell surface will be much easier since ROS do not need to cross the membrane. FAPdL5** requires a much lower light intensity than KillerRed or miniSOG, indicating that FAPdL5** might be a stronger oxidizing agent for faster polymerization. While both miniSOG and FAPdL5** could be robust general choices for photopolymerization, given the track record of miniSOG use in oxidative polymerization, we and others successfully tested miniSOG first in two concomitant GTCA papers near the end of 2022[74,75].

Going forward, to explore light-based patterning, 2D cell cultures may be used for optimizing writing/polymerization speed by tuning light intensity and composition of polymer precursor mixtures (e.g., aniline and pyrrole derivatives) with different oxidation potentials. Laser scanning along a line or other defined 3D trajectory in brain tissue may be used to generate long-range conductive pathways, which can be connected to implantable or surface electrodes for neural recording and modulation. To prevent crosstalk and current leakage from the assembled conductors, the conductive polymer wires may be selectively assembled with insulated coating of non-conductive polymers, both polymers created by light-mediated GTCA. Such an approach to regulate cellular activity may be considered a mode of *optogenetic GTCA* (**Fig. 3a**), since light

sensitivity is conferred in a genetically targeted way with the intent to control activity of specific cells– just as conventional optogenetics achieves with microbial opsins[61].

b. Mode 2: Light-sensitive proton pumps for pH-regulated GTCA

In photolithography, distinct from photo-initiated radical polymerizations, a common photoresist chemistry of SU-8 employs a light-induced acid generator to catalyze ring-opening reactions[76]. This important concept can be potentially translated to GTCA, where the targeted acid generator could be a genetically-encoded protein that changes juxtamembranous pH upon light delivery. Excellent candidates to assume this role would include members of the microbial rhodopsin family– specifically the subfamily of all-in-one, single-gene-encoded, light-driven proton pumps[77]. We previously found that optogenetic activation of proton-pumping rhodopsins will suffice to reduce extracellular juxtamembranous pH sufficiently to activate pH-dependent ion channels[78,79].

We have identified ring-opening reactions, such as tetrahydrofuran (THF) polymerization, that can be robustly catalyzed by acids[80] (**Fig. 3b**). Many avenues exist (as needed) for optimization of microbial rhodopsins, including tuning of light sensitivity, photocurrent magnitude, and kinetics, for specific GTCA applications including THF polymerization[81], leveraging the detailed structural and mechanistic information that has been assembled in recent years[77]. Of note, separate from triggering acid-catalyzed ring-opening polymerization before optimization, this approach will also provide opportunity for use of light-modulated pH changes to dope conductive polymers (**Fig. 3b**), a well-known method for increasing conductivity of conductive polymers by several orders of magnitude[82].

c. Mode 3: temperature-regulated GTCA

Over the past decade, a number of studies have used nanoparticle transducers to convert external fields into different forms of energy (light, heat, electrical, mechanical) that can modulate neural activity[83,84]. For example, upconverting nanoparticles (NPs) can convert external infrared or near-infrared (NIR) wavelengths into local emission of visible light; NIR light allows deeper tissue penetration, and the converted visible light can optogenetically activate neurons in deep brain regions without insertion of an optical fiber[85]. However, for direct neural control, upconversion may be not the most efficient method; modern ultrasensitive microbial rhodopsins (especially the fast channelrhodopsin ChRmine) allow fast deep optogenetic control even without upconversion[86].

Optothermal transducers, such as gold NPs[87], fuzzy graphene[88], and silicon structures[89,90], can convert light into local heat, increasing the temperature by up to 10 °C (which is sufficient to directly modulate membrane excitability, leading to depolarization and activation of neurons). In addition to using light as the energy input to generate heat, many studies have explored other relevant signals, such as magnetic fields and ultrasound waves. Specifically, magnetic control of neural activity has been achieved using magnetic NP heating of temperature-sensitive ion channels[91-93] (piezoelectric NPs have also been used to directly convert ultrasound waves into electricity to modulate neural activity[94,95]).

By selectively conjugating nanotransducers onto living neural membranes in the GTCA *ad cellula* regime, one can readily adapt these strategies for neural modulation with cell type specificity (**Fig. 3c**). In addition, temperature represents a critical condition for many subsequent reactions. Specifically, enzymes have the highest catalytic activity within narrow temperature ranges. For example, the optimum temperature of HRP is about 35 °C[96]. Below the optimum

temperature, catalytic activity increases roughly linearly with temperature. Above the optimum temperature, activity decreases significantly, with only 40 % activity retained at 45 °C. Accordingly, optothermal nanotransducers and magnetic NPs could be used to selectively control polymerization.

d. Mode 4: optically-targeted gene expression for GTCA control

To photopattern functional materials in a genetically targeted fashion, yet another strategy may be to use light-regulated transcriptional promoters or enhancers to control the transcription of downstream GTCA-relevant genes[97], such as the HRP enzyme to modulate redox conditions. For example, a modified form of the Tet-ON system (a commonly-used chemically-regulated gene expression tool for mammalian cells) has been developed, rendered the system responsive to blue light[98]. The original Tet-ON consists of a modified transcription factor (TetR) fused to a transcription activation domain, which can recognize and drive gene expression from a specific DNA sequence (the tetracycline-responsive promoter element or TRE); however, transcription occurs only in the presence of the small molecule doxycycline (Dox), which binds TetR and allows association and transcription activation at the TRE[99].

In the modified photoactivatable Tet-ON system[98], the transcription activation domain and the TetR are separately fused to the cryptochrome 2 (Cry2) photoreceptor and its specific binding protein cryptochrome-interacting basic helix-loop-helix 1 (CIB1), respectively (**Fig. 3d**). Upon blue light exposure, the TetR specifically binds to the transcription activation domain through the Cry2-CIB1 light-inducible binding switch; expression of the gene of interest can thus be tightly regulated under the control of both light and Dox. The same principle has also been used to achieve light regulation of the Gal4/UAS gene expression system in mammalian cells[100]. Notably, many

other light-inducible dimerization pairs could potentially improve this system, such as phytochrome B (PhyB)–phytochrome-interacting factor (PIF), which has significantly faster kinetics and operates at long wavelength light[101].

Using such light controlled gene expression, an early form of photolithography with bacterial cells has been achieved by optically regulating cell adhesion to substrates[102,103]. Now with the wide variety of light-regulated gene expression tools available[97], any GTCA synthetic reaction with a genetically-encoded catalyst component can be well-controlled.

IV. Applications from central nervous systems to peripheral nervous systems

From the beginning, the long-term vision for GTCA has been one of general applicability to any animal or biological system. In our first demonstration of GTCA, we achieved neural modulation in wild-type/non-transgenic mammalian (rodent) brains and the freely behaving *C. elegans* nervous system[15]. In this section, we describe several potential research and clinical application opportunities across non-mammalian and mammalian systems and tissues.

a. Genetically targeted 3D photolithography in zebrafish brains

For *in vivo* 3D photolithography-based exploration of neural circuits, both one-photon and two-photon high-resolution imaging and stimulation approaches may be used, including MultiMAP[104,105] and MultiSLM[62]. In MultiMAP, two-photon microscopy is used to perform brain-wide calcium imaging in live zebrafish, followed by cellular-level registration of the molecular identity in fixed brains. For fast integration of single-cell imaging and stimulation we developed a method called MultiSLM, for which we designed a high-pixel-density spatial light modulator (SLM) for high-fidelity near-infrared hologram generation. This technique enabled kilohertz 3D

read-write optogenetic access to large ensembles of single neurons (N > 1000) over millimeter spatial scales[62].

To develop versatile light-controlled polymerization while leveraging zebrafish-relevant tools such as MultiMAP and MultiSLM, a transgenic zebrafish line encoding miniSOG on all neuron membranes could be used to optimize polymerization parameters, including monomer type/concentration, light intensity, and writing speed with MultiSLM. For neural modulation, distinct fish lines restricting expression of miniSOG (for example to serotonergic neurons of the dorsal raphe[106]) alongside nuclear-localized Ca^{2+} activity reporter GCaMP6s in all neurons would be of substantial interest. MultiMAP[104] could then be used: to perform Ca^{2+} imaging of spontaneous neural activity from dorsal raphe, to identify the serotonergic cells, and to register molecularly-defined cell type with the Ca^{2+} activity map. A photopatterning approach could then be used to write a conductive neural lace connecting two or more structures– for example, along the habenular commissure connecting the left and right habenula (short range, 25 μm) and/or the pathway connecting habenula and raphe (long range, 250 μm) (**Fig. 4a**).

After patterning a conductive neural lace between brain regions, recurrent neural network (RNN) models[107,108] could be used to assess altered current flow in these arbitrarily-constructed neural networks *in situ*. RNN models provide an estimation of the effective strength and type (i.e., excitatory or inhibitory) of interactions both within and across regions, from experimentally-observed neural dynamics. In the constructed RNN models, current flow between the two regions therefore can be estimated before and after the patterning of the neural lace from the model's recapitulation of observed activity[107,108]. Higher currents, interaction weights, or synchronized firing between regions connected with the neural lace may be quantified; such measures are

important since the ability to precisely and stably strengthen or weaken specific projections across the brain is crucial for the meaningful control of brain states, dynamics, and behavior[77,109,110].

To further quantify neural-dynamics outcomes of GTCA-created structures at single-cell resolution, brain-wide single-cell influence mapping[111] may be applied to measure how firing of one neuron causally affects spiking in its downstream partners. A fish line expressing miniSOG, GCaMP, and a channelrhodopsin such as ChRmine[62,112] in all neurons may be used alongside MultiSLM to concurrently stimulate cells on or adjacent to the neural lace. By measuring responses across the whole brain, one can examine single-cell connectivity of targeted regions[109], and quantify potential on- and off-target effects of the neural lace alongside behavior.

b. Modulation of the mammalian peripheral nervous system

To explore application of GTCA in the mammalian peripheral nervous system, HRP-encoding viral vectors may be delivered to the sciatic nerve via intraneural injection (**Fig. 4b**), after which solutions containing polymer precursors may be injected to deposit conductive or insulating polymers. Before and after polymer assembly, modulation of responses to electrical stimulation (namely muscle voltage and leg movement) can be tracked, for example using chronically-implanted stretchable polymer-based electronics[113,114] which both delivers voltage pulses for electrical stimulation and records evoked action potentials. Alternatively, channelrhodopsin ChRmine[62,112] can be expressed in sciatic nerves for remote stimulation and activation threshold testing (**Fig. 4b**).

Cellular specificity of this approach is a key property and can be readily demonstrated, just as in the CNS. The sciatic nerve include sensory nerve fibers that are peripheral processes of neurons in the dorsal root ganglia, and motor fibers that are processes of anterior horn cells of the

spinal cord. Modern nerve stimulation approaches with implanted electrodes cannot readily distinguish these nerve types (to say nothing of their subtypes). GTCA could begin to provide physical and functional structures (that might ultimately be connected to external electronics) targeting specific cell type via selective expression of HRP on motor or sensory neurons in the sciatic nerve[115]. Cell type specificity can be readily assessed (with GTCA on motor neurons, changes of stimulation threshold for muscle twitching would be expected, while pain threshold changes would instead be expected in sensory neurons)[116].

c. Modulation of the mammalian central nervous system

Maintaining excitation-inhibition (E:I) balance is crucial for nervous system function[117,118], and altered balance (e.g. increased excitatory or decreased inhibitory inputs) has been implicated in the etiology of autism and schizophrenia. In our previous work, we demonstrated that acutely causing E:I balance changes in wild-type mice could elicit or correct deficits in social behavior[119]. We next used a transgenic mouse line lacking *CNTNAP2* that exhibits autism-like phenotypes[120]; in this knockout (KO) line, we optogenetically rescued deficits in social behavior via temporally-precise reduction in E:I balance in medial prefrontal cortex (either by optogenetically increasing excitability of inhibitory parvalbumin (PV) neurons, or by decreasing excitability of excitatory pyramidal neurons). In this work, we used excitatory (SSFO)[117] or inhibitory (SwiChR++)[121] step-function channelrhodopsins for highly light-sensitive optogenetic modulation.

Here, we note that increase of PV neuron excitability and/or decrease of excitatory pyramidal neuron excitability in the medial prefrontal cortex (mPFC) of *CNTNAP2* KO mice may also be achieved, now stably via GTCA (**Fig. 4c**) and compatible with social exploration testing as described previously[119]. In the simplest form of this experiment, deposition of PDAB on PV

neurons and/or PANI on pyramidal neurons could test for rescue of deficits in social interaction in autism-model *CNTNAP2* KO mice (**Fig. 4c**). More interestingly, the electrical connections forming and synchronizing[122] inhibitory neuronal networks could be supplemented by GTCA targeting gap junction networks linking PV neurons (**Fig. 4c**); in *CNTNAP2* KO mice where E:I balance is too high, synchronizing firing of PV neurons could restore balance along with natural brain and behavioral states.

V. Outlook

By integrating genetic methods with polymer chemistry and materials science, GTCA can be used to instruct specific living cells to guide assembly of functional materials. While the structural and functional complexity and plasticity of multicellular biological systems, such as the brain, represent a major challenge for interface design, GTCA recruits the molecular machinery of specific cells in living organisms to construct synthetic materials and assemble them into cell-specific functional interfaces. With these technological advances, we envision that GTCA will rapidly give rise to new seamless, precisely-targeted brain interfaces, and will lead to new therapeutic approaches in conjunction with the emerging field of bioelectronic medicine[123].

While our initial studies demonstrated the promise of GTCA, further studies are required to explore potential toxicity and long-term biocompatibility of synthesized materials, and the physiological impact of the assembled structures on living systems. Specifically,existing strategies for increasing biocompatibility of implantable neural interfaces may be adapted to GTCA. For example, biopolymer-based coatings are known to support neuronal adhesion and reduce inflammatory response to brain implants[124], GTCA might achieve the same effects through co-polymerization with peptides, such as extracellular matrix-derived materials. In addition,

biodegradable polymers, including collagen, chitosan, alginate, dextran, and silk, are often used as substrates for transient electronics[125,126]. These polymers can either be incorporated during *in situ* material assembly, or can provide a more biocompatible environment during reaction[127].

References

1 Acarón Ledesma, H. *et al.* An atlas of nano-enabled neural interfaces. *Nat. Nanotechnol.* **14**, 645-657 (2019).

2 Chen, R., Canales, A. & Anikeeva, P. Neural recording and modulation technologies. *Nat. Rev. Mater.* **2**, 16093 (2017).

3 Rivnay, J., Wang, H., Fenno, L., Deisseroth, K. & Malliaras, G. G. Next-generation probes, particles, and proteins for neural interfacing. *Sci. Adv.* **3**, e1601649 (2017).

4 Zhang, A., Lee, J.-H. & Lieber, C. M. Nanowire-enabled bioelectronics. *Nano Today* **38**, 101135 (2021).

5 Luan, L. *et al.* Recent advances in electrical neural interface engineering: minimal invasiveness, longevity, and scalability. *Neuron* **108**, 302-321 (2020).

6 Zhang, M., Tang, Z., Liu, X. & Van der Spiegel, J. Electronic neural interfaces. *Nat. Electron.* **3**, 191-200 (2020).

7 Song, E., Li, J., Won, S. M., Bai, W. & Rogers, J. A. Materials for flexible bioelectronic systems as chronic neural interfaces. *Nat. Mater.* **19**, 590-603 (2020).

8 Woods, G. A., Rommelfanger, N. J. & Hong, G. Bioinspired materials for in vivo bioelectronic neural interfaces. *Matter* **3**, 1087-1113 (2020).

9 Berggren, M., Głowacki, E. D., Simon, D. T., Stavrinidou, E. & Tybrandt, K. In vivo organic bioelectronics for neuromodulation. *Chem. Rev.* **122**, 4826-4846 (2022).

10 Tang, X., Shen, H., Zhao, S., Li, N. & Liu, J. Flexible brain–computer interfaces. *Nat. Electron.* **6**, 109-118 (2023).

11 Tang, T.-C. *et al.* Materials design by synthetic biology. *Nat. Rev. Mater.* **6**, 332-350 (2021).

12 Burgos-Morales, O. *et al.* Synthetic biology as driver for the biologization of materials sciences. *Mater. Today Bio* **11**, 100115 (2021).

13 Brophy, J. A. N. & Voigt, C. A. Principles of genetic circuit design. *Nat. Methods* **11**, 508-520 (2014).

14 Ouyang, L., Shaw, C. L., Kuo, C.-c., Griffin, A. L. & Martin, D. C. In vivo polymerization of poly (3, 4-ethylenedioxythiophene) in the living rat hippocampus does not cause a significant loss of performance in a delayed alternation task. *J. Neural Eng.* **11**, 026005 (2014).

15 Liu, J. *et al.* Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. *Science* **367**, 1372-1376 (2020).

16 Zhang, A. *et al.* In situ genetically targeted chemical assembly of polymers on living neuronal membranes. *Preprint at bioRxiv*, https://doi.org/10.1101/2022.12.27.521974 (2022).

17 Dai, Y. *et al.* Oxidative polymerization in living cells. *J. Am. Chem. Soc.* **143**, 10709-10717 (2021).

18 Pieszka, M. *et al.* Controlled supramolecular assembly inside living cells by sequential multistaged chemical reactions. *J. Am. Chem. Soc.* **142**, 15780-15789 (2020).

19 Zhang, Y. *et al.* Controlled intracellular polymerization for cancer treatment. *JACS Au* **2**, 579-589 (2022).

20 Guimard, N. K., Gomez, N. & Schmidt, C. E. Conducting polymers in biomedical engineering. *Prog. Polym. Sci.* **32**, 876-921 (2007).

21 Tabba, H. D. & Smith, K. M. Anodic oxidation potentials of substituted pyrroles: derivation and analysis of substituent partial potentials. *J. Org. Chem.* **49**, 1870-1875 (1984).
22 Berlin, A., Pagani, G. A., Sannicolò, F., Schiavon, G. & Zotti, G. Monomer tailoring to control the redox potentials of conductive polyheterocycles. *Polym.* **32**, 1841-1842 (1991).
23 Tan, Y. & Ghandi, K. Kinetics and mechanism of pyrrole chemical polymerization. *Synth. Met.* **175**, 183-191 (2013).
24 Gaupp, C. L. *et al.* Poly(3,4-ethylenedioxypyrrole): Organic electrochemistry of a highly stable electrochromic polymer. *Macromolecules* **33**, 1132-1133 (2000).
25 Sönmez, G., Schottland, P., Zong, K. & Reynolds, J. R. Highly transmissive and conductive poly[(3,4-alkylenedioxy)pyrrole-2,5-diyl] (PXDOP) films prepared by air or transition metal catalyzed chemical oxidation. *J. Mater. Chem.* **11**, 289-294 (2001).
26 Kuwabata, S., Ito, S. & Yoneyama, H. Copolymerization of pyrrole and thiophene by electrochemical oxidation and electrochemical behavior of the resulting copolymers. *J. Electrochem. Soc.* **135**, 1691 (1988).
27 Tewari, A. *et al.* Soybean peroxidase catalyzed enzymatic synthesis of pyrrole/EDOT copolymers. *Macromol. Chem. Phys.* **211**, 1610-1617 (2010).
28 John, R. & Wallace, G. Doping-dedoping of polypyrrole: a study using current-measuring and resistance-measuring techniques. *J. Electroanal. Chem.* **354**, 145-160 (1993).
29 Cruz-Silva, R. *et al.* Biocatalytic synthesis of polypyrrole powder, colloids, and films using horseradish peroxidase. *J. Colloid Interface Sci.* **328**, 263-269 (2008).
30 Song, H.-K., Lee, E. J. & Oh, S. M. Electrochromism of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) incorporated into conducting polymer as a dopant. *Chem. Mat.* **17**, 2232-2233 (2005).
31 Yoo, J. E. *et al.* Improving the electrical conductivity of polymer acid-doped polyaniline by controlling the template molecular weight. *J. Mater. Chem.* **17**, 1268-1275 (2007).
32 Bayer, C. L., Trenchard, I. J. & Peppas, N. A. Analyzing polyaniline-poly(2-acrylamido-2-methylpropane sulfonic acid) biocompatibility with 3T3 fibroblasts. *J. Biomater. Sci. Polym. Ed.* **21**, 623-634 (2010).
33 Zhang, W., Yang, F. K., Pan, Z., Zhang, J. & Zhao, B. Bio-inspired dopamine functionalization of polypyrrole for improved adhesion and conductivity. *Macromol. Rapid. Comm.* **35**, 350-354 (2014).
34 Xie, C. *et al.* Electroresponsive and cell-affinitive polydopamine/polypyrrole composite microcapsules with a dual-function of on-demand drug delivery and cell stimulation for electrical therapy. *NPG Asia Mater.* **9**, e358 (2017).
35 Chalmers, E., Lee, H., Zhu, C. & Liu, X. Increasing the conductivity and adhesion of polypyrrole hydrogels with electropolymerized polydopamine. *Chem. Mat.* **32**, 234-244 (2020).
36 Bedard, K. & Krause, K.-H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* **87**, 245-313 (2007).
37 Ma, M. W. *et al.* NADPH oxidase in brain injury and neurodegenerative disorders. *Mol. Neurodegener.* **12**, 1-28 (2017).
38 Suh, S. W. *et al.* Glucose and NADPH oxidase drive neuronal superoxide formation in stroke. *Ann. Neurol.* **64**, 654-663 (2008).

39 Bankar, S. B., Bule, M. V., Singhal, R. S. & Ananthanarayan, L. Glucose oxidase — An overview. *Biotechnol. Adv.* **27**, 489-501 (2009).

40 Mayer, A. M. & Staples, R. C. Laccase: new functions for an old enzyme. *Phytochemistry* **60**, 551-565 (2002).

41 Kausaite, A., Ramanaviciene, A. & Ramanavicius, A. Polyaniline synthesis catalysed by glucose oxidase. *Polym.* **50**, 1846-1851 (2009).

42 Karamyshev, A. V., Shleev, S. V., Koroleva, O. V., Yaropolov, A. I. & Sakharov, I. Y. Laccase-catalyzed synthesis of conducting polyaniline. *Enzyme Microb. Technol.* **33**, 556-564 (2003).

43 Ramanavicius, A., Kausaite, A., Ramanaviciene, A., Acaite, J. & Malinauskas, A. Redox enzyme – glucose oxidase – initiated synthesis of polypyrrole. *Synth. Met.* **156**, 409-413 (2006).

44 Song, H.-K. & Palmore, G. T. R. Conductive polypyrrole via enzyme catalysis. *J. Phys. Chem. B* **109**, 19278-19287 (2005).

45 Porto de Souza Vandenberghe, L. *et al.* in *Biomass, Biofuels, Biochemicals* (eds Sudhir P. Singh *et al.*) 11-30 (Elsevier, 2020).

46 Prescher, J. A. & Bertozzi, C. R. Chemistry in living systems. *Nat. Chem. Biol.* **1**, 13-21 (2005).

47 Chyan, W. & Raines, R. T. Enzyme-activated fluorogenic probes for live-cell and in vivo imaging. *ACS Chem. Biol.* **13**, 1810-1823 (2018).

48 Yang, Y., Lee, P. & Sternson, S. M. Cell type-specific pharmacology of NMDA receptors using masked MK801. *eLife* **4**, e10206 (2015).

49 Saxon, E. & Bertozzi, C. R. Cell surface engineering by a modified Staudinger reaction. *Science* **287**, 2007-2010 (2000).

50 Prescher, J. A., Dube, D. H. & Bertozzi, C. R. Chemical remodelling of cell surfaces in living animals. *Nature* **430**, 873-877 (2004).

51 Cioce, A. *et al.* Cell-specific bioorthogonal tagging of glycoproteins. *Nat. Commun.* **13**, 6237 (2022).

52 Schumann, B. *et al.* Bump-and-hole engineering identifies specific substrates of glycosyltransferases in living cells. *Mol. Cell* **78**, 824-834.e15 (2020).

53 Fan, X. *et al.* Cell-type-specific labeling and profiling of glycans in living mice. *Nat. Chem. Biol.* **18**, 625-633 (2022).

54 Chin, J. W. Expanding and reprogramming the genetic code. *Nature* **550**, 53-60 (2017).

55 Tian, L. *et al.* Selective esterase–ester pair for targeting small molecules with cellular specificity. *Proc. Natl. Acad. Sci. USA* **109**, 4756-4761 (2012).

56 Zakeri, B. *et al.* Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proc. Natl. Acad. Sci. USA* **109**, E690-E697 (2012).

57 Grenier, V., Daws, B. R., Liu, P. & Miller, E. W. Spying on neuronal membrane potential with genetically targetable voltage indicators. *J. Am. Chem. Soc.* **141**, 1349-1358 (2019).

58 Bedbrook, Claire N. *et al.* Genetically encoded Spy peptide fusion system to detect plasma membrane-localized proteins in vivo. *Chem. Biol.* **22**, 1108-1121 (2015).

59 Veggiani, G. *et al.* Programmable polyproteams built using twin peptide superglues. *Proc. Natl. Acad. Sci. USA* **113**, 1202-1207 (2016).

60 Prakash, R. *et al.* Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation. *Nat. Methods* **9**, 1171-1179 (2012).

61 Deisseroth, K. Optogenetics: 10 years of microbial opsins in neuroscience. *Nat. Neurosci.* **18**, 1213-1225 (2015).
62 Marshel, J. H. *et al.* Cortical layer–specific critical dynamics triggering perception. *Science* **365**, eaaw5202 (2019).
63 Wojtovich, A. P. & Foster, T. H. Optogenetic control of ROS production. *Redox Biol.* **2**, 368-376 (2014).
64 Balena, A., Bianco, M., Pisanello, F. & De Vittorio, M. Recent advances on high-speed and holographic two-photon direct laser writing. *Adv. Funct. Mater.*, 2211773 (2023).
65 Bulina, M. E. *et al.* A genetically encoded photosensitizer. *Nat. Biotechnol.* **24**, 95-99 (2006).
66 Zhou, Z., Song, J., Nie, L. & Chen, X. Reactive oxygen species generating systems meeting challenges of photodynamic cancer therapy. *Chem. Soc. Rev.* **45**, 6597-6626 (2016).
67 Rodríguez-Pulido, A. *et al.* Assessing the potential of photosensitizing flavoproteins as tags for correlative microscopy. *Chem. Commun.* **52**, 8405-8408 (2016).
68 Shu, X. *et al.* A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.* **9**, e1001041 (2011).
69 Trewin, A. J. *et al.* Light-induced oxidant production by fluorescent proteins. *Free Radic. Biol. Med.* **128**, 157-164 (2018).
70 Westberg, M., Holmegaard, L., Pimenta, F. M., Etzerodt, M. & Ogilby, P. R. Rational design of an efficient, genetically encodable, protein-encased singlet oxygen photosensitizer. *J. Am. Chem. Soc.* **137**, 1632-1642 (2015).
71 Westberg, M., Bregnhøj, M., Etzerodt, M. & Ogilby, P. R. No photon wasted: an efficient and selective singlet oxygen photosensitizing protein. *J. Phys. Chem. B* **121**, 9366-9371 (2017).
72 Makhijani, K. *et al.* Precision optogenetic tool for selective single-and multiple-cell ablation in a live animal model system. *Cell Chem. Biol.* **24**, 110-119 (2017).
73 He, J. *et al.* A genetically targetable near-infrared photosensitizer. *Nat. Methods* **13**, 263-268 (2016).
74 Zhang, A., Kadur, C. S., Ramakrishnan, C., Bao, Z. & Deisseroth, K. Genetically-encoded photosensitizers enable light-controlled polymerization on living neuronal membranes. *Preprint at bioRxiv*, https://doi.org/10.1101/2022.12.27.521977 (2022).
75 Sessler, C. D. *et al.* Optogenetic polymerization and assembly of electrically functional polymers for modulation of single-neuron excitability. *Sci. Adv.* **8**, eade1136 (2022).
76 del Campo, A. & Greiner, C. SU-8: a photoresist for high-aspect-ratio and 3D submicron lithography. *J. Micromech. Microeng* **17**, R81 (2007).
77 Deisseroth, K. From microbial membrane proteins to the mysteries of emotion. *Cell* **184**, 5279-5285 (2021).
78 Ferenczi, E. A. *et al.* Optogenetic approaches addressing extracellular modulation of neural excitability. *Sci. Rep.* **6**, 23947 (2016).
79 Mattis, J. *et al.* Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat. Methods* **9**, 159-172 (2012).
80 Pruckmayr, G. & Wu, T. K. Polymerization of tetrahydrofuran by proton acids. *Macromolecules* **11**, 662-668 (1978).
81 Deisseroth, K. & Hegemann, P. The form and function of channelrhodopsin. *Science* **357**, eaan5544 (2017).

82 Hatchett, D. W., Josowicz, M. & Janata, J. Acid doping of polyaniline: Spectroscopic and electrochemical studies. *J. Phys. Chem. B* **103**, 10992-10998 (1999).
83 Li, X. *et al.* Nanotransducers for wireless neuromodulation. *Matter* **4**, 1484-1510 (2021).
84 Liu, Y., Yi, Z., Yao, Y., Guo, B. & Liu, X. Noninvasive manipulation of ion channels for neuromodulation and theranostics. *Acc. Mater. Res.* **3**, 247-258 (2022).
85 Chen, S. *et al.* Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics. *Science* **359**, 679-684 (2018).
86 Chen, R. *et al.* Deep brain optogenetics without intracranial surgery. *Nat. Biotechnol.* **39**, 161-164 (2021).
87 Carvalho-de-Souza, João L. *et al.* Photosensitivity of neurons enabled by cell-targeted gold nanoparticles. *Neuron* **86**, 207-217 (2015).
88 Rastogi, S. K. *et al.* Remote nongenetic optical modulation of neuronal activity using fuzzy graphene. *Proc. Natl. Acad. Sci. USA* **117**, 13339-13349 (2020).
89 Jiang, Y. *et al.* Heterogeneous silicon mesostructures for lipid-supported bioelectric interfaces. *Nat. Mater.* **15**, 1023-1030 (2016).
90 Jiang, Y. *et al.* Rational design of silicon structures for optically controlled multiscale biointerfaces. *Nat. Biomed. Eng.* **2**, 508-521 (2018).
91 Huang, H., Delikanli, S., Zeng, H., Ferkey, D. M. & Pralle, A. Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat. Nanotechnol.* **5**, 602-606 (2010).
92 Chen, R., Romero, G., Christiansen, M. G., Mohr, A. & Anikeeva, P. Wireless magnetothermal deep brain stimulation. *Science* **347**, 1477-1480 (2015).
93 Sebesta, C. *et al.* Subsecond multichannel magnetic control of select neural circuits in freely moving flies. *Nat. Mater.* **21**, 951-958 (2022).
94 Marino, A. *et al.* Piezoelectric nanoparticle-assisted wireless neuronal stimulation. *ACS Nano* **9**, 7678-7689 (2015).
95 Kim, T. *et al.* Deep brain stimulation by blood–brain-barrier-crossing piezoelectric nanoparticles generating current and nitric oxide under focused ultrasound. *Nat. Biomed. Eng.* (2022).
96 Jin, W., Wang, R. & Huang, X. Horseradish peroxidase-catalyzed oxidative polymerization of aniline in bicontinuous microemulsion stabilized by AOT/SDS. *J. Mol. Liq.* **302**, 112529 (2020).
97 Yamada, M., Nagasaki, S. C., Ozawa, T. & Imayoshi, I. Light-mediated control of Gene expression in mammalian cells. *Neurosci. Res.* **152**, 66-77 (2020).
98 Yamada, M., Suzuki, Y., Nagasaki, S. C., Okuno, H. & Imayoshi, I. Light control of the Tet gene expression system in mammalian cells. *Cell Rep.* **25**, 487-500.e6 (2018).
99 Gossen, M. *et al.* Transcriptional activation by tetracyclines in mammalian cells. *Science* **268**, 1766-1769 (1995).
100 Yamada, M., Nagasaki, S. C., Suzuki, Y., Hirano, Y. & Imayoshi, I. Optimization of light-inducible Gal4/UAS gene expression system in mammalian cells. *iScience* **23**, 101506 (2020).
101 Uda, Y. *et al.* Efficient synthesis of phycocyanobilin in mammalian cells for optogenetic control of cell signaling. *Proc. Natl. Acad. Sci. USA* **114**, 11962-11967 (2017).
102 Jin, X. & Riedel-Kruse, I. H. Biofilm Lithography enables high-resolution cell patterning via optogenetic adhesin expression. *Proc. Natl. Acad. Sci. USA* **115**, 3698-3703 (2018).

103 Zhao, F. *et al.* Light-induced patterning of electroactive bacterial biofilms. *ACS Synth. Biol.* **11**, 2327-2338 (2022).
104 Lovett-Barron, M. *et al.* Ancestral circuits for the coordinated modulation of brain state. *Cell* **171**, 1411-1423. e17 (2017).
105 Lovett-Barron, M. *et al.* Multiple convergent hypothalamus–brainstem circuits drive defensive behavior. *Nat. Neurosci.* **23**, 959-967 (2020).
106 Yokogawa, T., Hannan, M. C. & Burgess, H. A. The dorsal raphe modulates sensory responsiveness during arousal in zebrafish. *J. Neurosci.* **32**, 15205-15215 (2012).
107 Rajan, K., Harvey, C. D. & Tank, D. W. Recurrent network models of sequence generation and memory. *Neuron* **90**, 128-142 (2016).
108 Perich, M. G. *et al.* Inferring brain-wide interactions using data-constrained recurrent neural network models. *Preprint at bioRxiv*, https://doi.org/10.1101/2020.12.18.423348 (2020).
109 Andalman, A. S. *et al.* Neuronal dynamics regulating brain and behavioral state transitions. *Cell* **177**, 970-985.e20 (2019).
110 Deisseroth, K. Circuit dynamics of adaptive and maladaptive behaviour. *Nature* **505**, 309-317 (2014).
111 Chettih, S. N. & Harvey, C. D. Single-neuron perturbations reveal feature-specific competition in V1. *Nature* **567**, 334-340 (2019).
112 Kishi, K. E. *et al.* Structural basis for channel conduction in the pump-like channelrhodopsin ChRmine. *Cell* **185**, 672-689.e23 (2022).
113 Liu, Y. *et al.* Morphing electronics enable neuromodulation in growing tissue. *Nat. Biotechnol.* **38**, 1031-1036 (2020).
114 Li, T. L. *et al.* Stretchable mesh microelectronics for the biointegration and stimulation of human neural organoids. *Biomaterials.* **290**, 121825 (2022).
115 Ward, P. J. & English, A. W. Optical stimulation and electrophysiological analysis of regenerating peripheral axons. *Bio Protoc.* **9**, e3281 (2019).
116 Yalcin, I. *et al.* The sciatic nerve cuffing model of neuropathic pain in mice. *J. Vis. Exp.*, e51608 (2014).
117 Yizhar, O. *et al.* Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **477**, 171-178 (2011).
118 Tatti, R., Haley, M. S., Swanson, O. K., Tselha, T. & Maffei, A. Neurophysiology and regulation of the balance between excitation and inhibition in neocortical circuits. *Biol. Psychiatry* **81**, 821-831 (2017).
119 Selimbeyoglu, A. *et al.* Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. *Sci. Transl. Med.* **9**, eaah6733 (2017).
120 Peñagarikano, O. *et al.* Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell* **147**, 235-246 (2011).
121 Berndt, A. *et al.* Structural foundations of optogenetics: Determinants of channelrhodopsin ion selectivity. *Proc. Natl. Acad. Sci. USA* **113**, 822-829 (2016).
122 Pernelle, G., Nicola, W. & Clopath, C. Gap junction plasticity as a mechanism to regulate network-wide oscillations. *PLoS Comput. Biol.* **14**, e1006025 (2018).
123 Patel, S. R. & Lieber, C. M. Precision electronic medicine in the brain. *Nat. Biotechnol.* **37**, 1007-1012 (2019).

124 Redolfi Riva, E. & Micera, S. Progress and challenges of implantable neural interfaces based on nature-derived materials. *Bioelectron. Med.* **7**, 6 (2021).
125 Shim, J.-S., Rogers, J. A. & Kang, S.-K. Physically transient electronic materials and devices. *Mater. Sci. Eng., R* **145**, 100624 (2021).
126 Irimia-Vladu, M. "Green" electronics: biodegradable and biocompatible materials and devices for sustainable future. *Chem. Soc. Rev.* **43**, 588-610 (2014).
127 Qin, Y. *et al.* Harnessing oxidative microenvironment for in vivo synthesis of subcellular conductive polymer microesicles enhances nerve reconstruction. *Nano Lett.* **22**, 3825-3831 (2022).
128 Williams, D. C. *et al.* Rapid and permanent neuronal inactivation in vivo via subcellular generation of reactive oxygen with the use of KillerRed. *Cell Rep.* **5**, 553-563 (2013).
129 Qi, Y. B., Garren, E. J., Shu, X., Tsien, R. Y. & Jin, Y. Photo-inducible cell ablation in Caenorhabditis elegans using the genetically encoded singlet oxygen generating protein miniSOG. *Proc. Natl. Acad. Sci. USA* **109**, 7499-7504 (2012).
130 Ng, J. *et al.* Genetically targeted 3D visualisation of Drosophila neurons under electron microscopy and X-Ray microscopy using miniSOG. *Sci. Rep.* **6**, 38863 (2016).
131 Liang, P., Kolodieznyi, D., Creeger, Y., Ballou, B. & Bruchez, M. P. Subcellular singlet oxygen and cell death: Location matters. *Front. Chem.* **8**, 1045 (2020).
132 Xie, W. *et al.* Chemoptogenetic ablation of neuronal mitochondria in vivo with spatiotemporal precision and controllable severity. *elife* **9**, e51845 (2020).

Acknowledgements

The Keck Foundation supported development of light-initiated polymerization and applications in living neural networks. The National Science Foundation Future Manufacturing Program grant (award no. 2037164) supported chemical method development for GTCA. Both grants were awarded to K.D. and Z.B. based on the original GTCA concept and next-generation methods described here[15]. A.Z. acknowledges support from American Heart Association (AHA) (award no. 23POST1018301). K.Y.L. acknowledges support from the Stanford ChEM-H Chemistry/Biology Interface Predoctoral Training Program (NIH 5T32GM120007) and Bio-X Bowes Fellowship.

Competing interests

All techniques and protocols are freely available to the academic community, and the authors provide free training in GTCA methods at Stanford in workshops that can be accessed online (https://web.stanford.edu/group/dlab/optogenetics/oil.html). Z.B. and K.D. are coinventors of the GTCA concept used here, in IP filed and owned by Stanford University.

Key points

- Genetically targeted chemical assembly (GTCA) uses cell-specific genetic information to guide the assembly of functional materials *in situ.*
- GTCA toolbox can be expanded through specific chemical processes involving novel monomers, catalysts, and reaction regimes, and alternative *ad cellula* approach that allows *ex situ* attachment of pre-synthesized materials.
- Potential diverse new GTCA-compatible reaction conditions can be imposed through modulation of light, pH, heat, and other signals.

- The broad GTCA concept can be applied to both research and clinical settings, including the central and peripheral nervous systems.

Author contributions

A.Z., Z.B., and K.D. wrote the manuscript with edits from all authors. Z.B. and K.D. supervised all aspects of the work. All authors approved the final version of the manuscript.

Table 1 Genetically encoded photosensitizer for photopolymerization

Protein	MW (kDa)	Ex/Em (nm)	Demonstrated applications	Cell ablation dose	Advantages	Disadvantages
KillerRed	~27*2 Dimer[65]	585/610[65]	Cell ablation[66,69]	153 J cm^{-2} in C. elegans[128]	Expression tested in transgenic *C. elegans*, *Drosophila* and zebrafish, and mouse retina with AAV[66,69]	Cannot polymerize DAB[67]
miniSOG	~14 Monomer[68]	448/528[68]	Cell ablation Polymerization of 3,3 - Diaminobenzidine (DAB)[66,69]	Cell ablation[129] 280 J cm^{-2} DAB polymerization 9.72 J cm^{-2} in solution[67] 120 J cm^{-2} in Drosophila[130]	Can polymerize DAB, tested in multiple reports[66,69] Expression tested in transgenic *C. elegans*, *Drosophila* and zebrafish, and mouse brain[66,69] Small size[68] Mutants of miniSOG are reported to increase $^1O_2$ production by ~10x[67,70-72]	The short excitation wavelength might cause phototoxicity
FAP dL5**	~25 Monomer[73]	669/705	Cell ablation	~7 J cm^{-2} [131]	Better tissue penetration Expression tested in transgenic zebrafish[132] Efficient energy conversion	Requires 30min-3h incubation in iodine-substituted dye before adding monomer

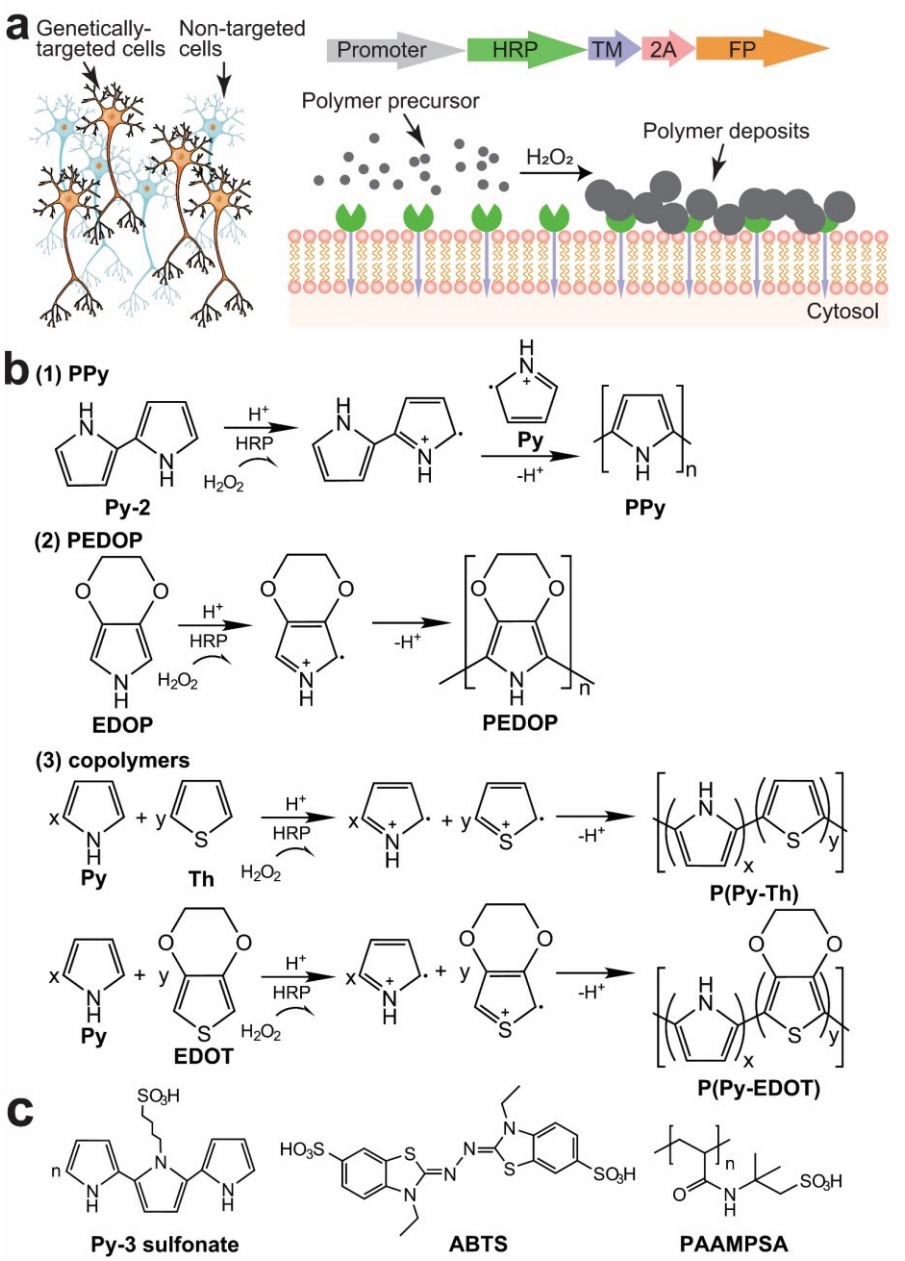

Figure 1 Genetically targeted chemical assembly (GTCA) of polymers *de cellula* on living cellular membranes: localization and polymerization schemata for pyrrole derivatives. (**a**) *Left*, living systems containing genetically-targeted (orange) and non-genetically-targeted (light blue) cells. *Top right*, DNA backbone for expressing membrane-displayed HRP. The construct is composed of (in one instantiation) a promoter for targeting specific cell types, followed by horseradish peroxidase (HRP), a transmembrane (TM) domain as the membrane targeting anchor, 2A self-cleaving peptides, and a fluorescent protein (FP). The targeted cells are expected to express membrane-displayed HRP and cytosolic FP. *Bottom right*, HRP/H_2O_2-catalyzed polymerization is designed to occur specifically on the membrane of enzyme-targeted cells. Polymer precursors form dark-colored aggregates deposited on the cell surface. (**b**) HRP-mediated polymerization of (1) polypyrrole (PPy), (2) poly(3,4-ethylenedioxypyrrole) (PEDOP), and (3) copolymer of pyrrole-thiophene (P(Py-Th)) and pyrrole-3,4-ethylenedioxythiophene (P(Py-EDOT)). (**c**) Structures of representative doping agents that may be incorporated during polymerization to increase electrical conductivity of the resulting polymers. *Left*, sulfonate-containing pyrrole trimer. *Middle*, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). *Right*, poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAAMPSA).

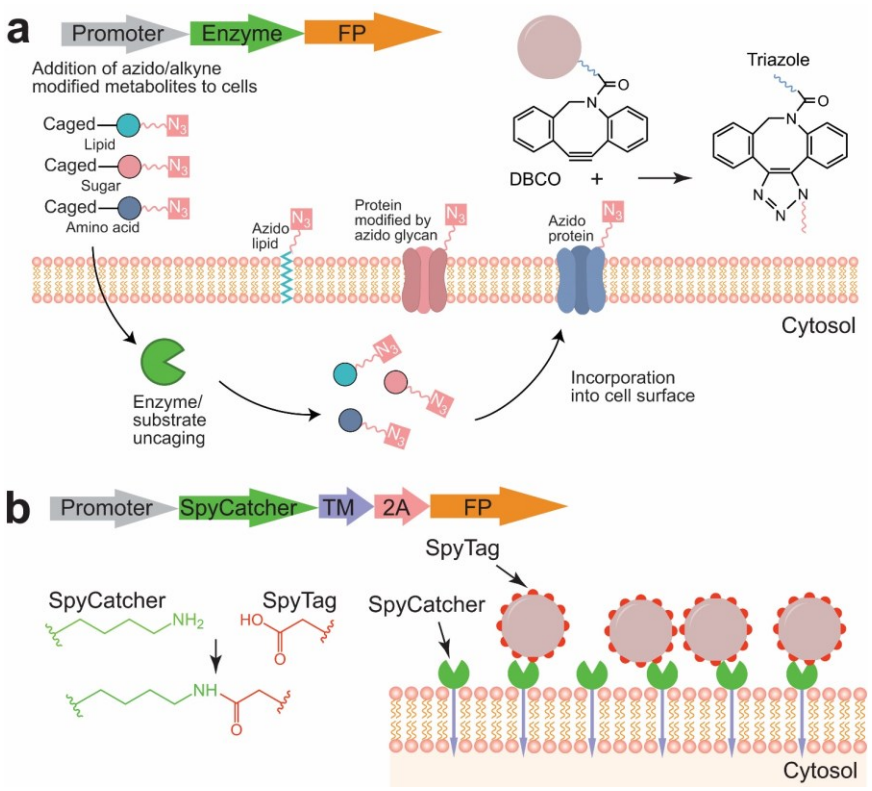

Figure 2 Genetically enabled *ad cellula* conjugation of pre-synthesized materials on living cell membranes. (**a**) *Top*, DNA backbone for expressing cytosolic uncaging enzyme with a promoter for targeting specific cell types. *Bottom*, Caged metabolites such as lipids, monosaccharides, amino acids could be internalized by cells and uncaged by specific intracellular enzymes. Unmasked metabolites could then be processed and incorporated into the lipidome, proteome and glycome. The unmasked azido sugars modify the glycocalyx layer (including glycoproteins and glycolipids) on the cell surface with azide ($-N_3$). Materials functionalized with DBCO groups form stable triazole with the surface azide. (**b**) *Top*, DNA backbone for expressing membrane-displayed SpyCatcher with a promoter for targeting specific cell types. SpyCatcher is anchored on the membrane surface with a transmembrane (TM) domain. *Bottom*, SpyCatcher anchored on the cell membrane enables extracellular conjugation of SpyTag-modified materials.

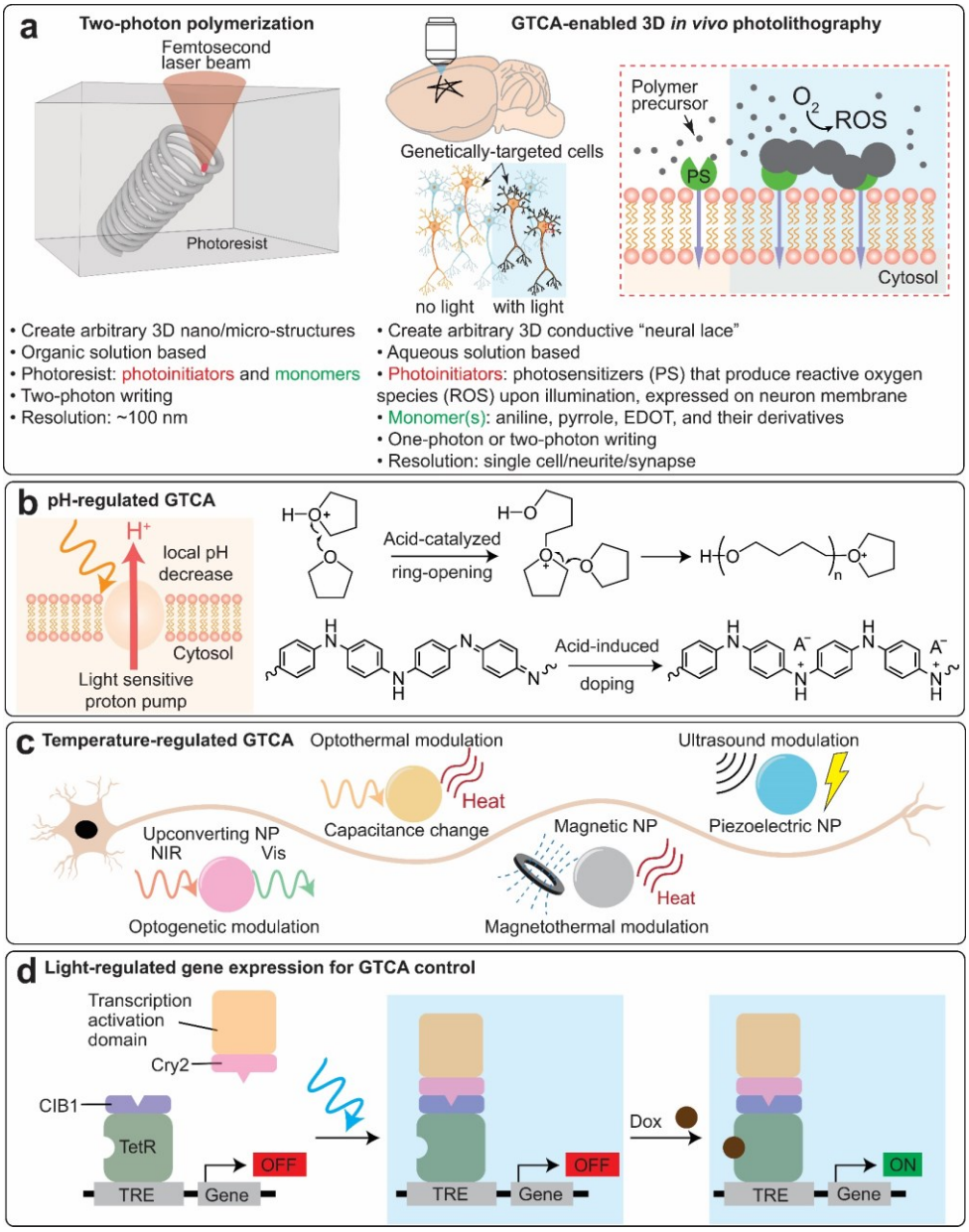

Figure 3 Exploring GTCA mechanisms: genetically-targeted reaction conditions. (**a**) Optogenetic GTCA (mode 1): genetically targeted photosensitizers and patterning of functional GTCA materials. Comparison of two-photon polymerization (*left*) and genetically targeted *in vivo* 3D photolithography (*right*). Red and green text colors show homologous components across fields. (**b**) Optogenetic GTCA (mode 2): light-mediated pH change as a genetically-targetable reaction condition. *Left*, the working principle of microbial opsin gene-encoded light-sensitive proton pumps, such as eArch3.0 activated with 560-nm light[61,79]. *Right*, light induced pH change can trigger ring-opening polymerization of THF and/or doping of conductive polymers. (**c**) Optogenetic GTCA (mode 3): light-mediated temperature change as a genetically-targetable reaction condition. Shown is neuron modulation by conjugated nanotransducers capable of converting different energy modalities (including light) into temperature. The "optothermal" mode can be complemented by magnetic and acoustic modes of temperature targeting. (**d**) Optogenetic GTCA (mode 4): light-mediated expression of general reaction modulators. A light-regulated Tet-ON gene expression system. Cry2-CIB1 dimer formation between the transcription factor (TetR) and the transcription activation domain can be induced by exposure to blue light. Genetically-targeted expression of any downstream gene (any reaction modulator) can then be controlled with systemic Dox application.

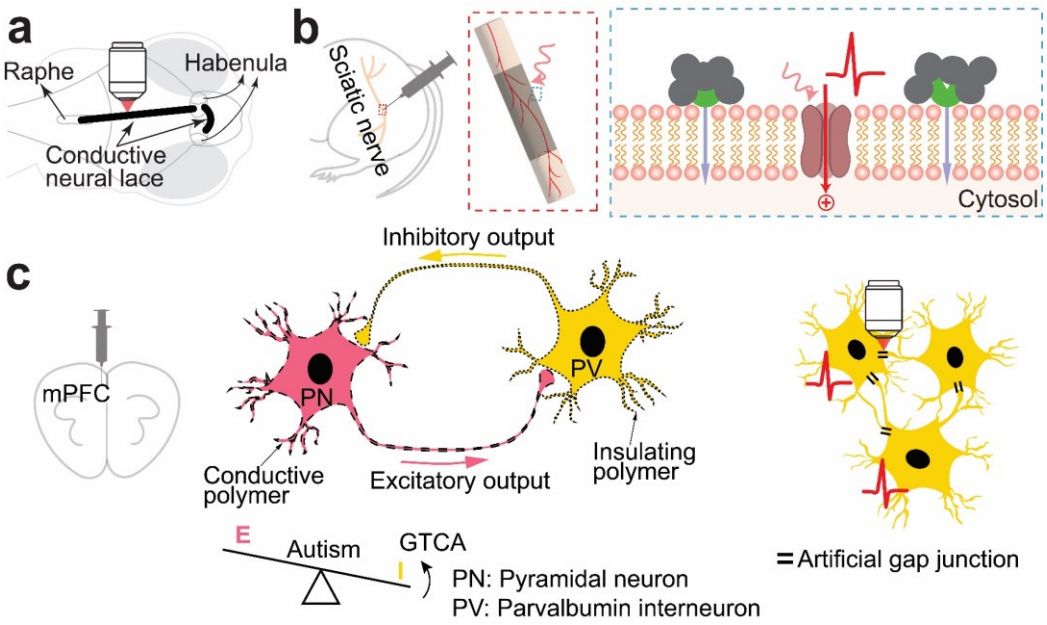

Figure 4 Potential applications from central to peripheral nervous systems. **(a)** Genetically targeted 3D photolithography in zebrafish brains. In a transgenic zebrafish line encoding miniSOG on all neuron membranes, MultiSLM may be used to pattern conductive neural lace connecting structures for which net activity/current flow is thought to be behaviorally-relevant, such as the left and right habenula and/or the habenula and raphe. (**b**) Peripheral nervous system modulation in mice. *Left*, Injection of HRP-encoding virus and polymer precursor solutions into mouse sciatic nerves. *Right*, polymer deposition modulates stimulation threshold, either electrical stimulation or as probed with optogenetics (red wave). (**c**) Exploring mouse models of autism. *Left*, injection of HRP-encoding virus and polymer precursor solutions into mPFC in autism-model mice, e.g. those lacking CNTNAP2. *Middle*, E:I balance between synaptic excitation is mediated in part by excitatory pyramidal and inhibitory PV neurons, and may be modulated (or even stably corrected) by properly targeted GTCA. *Right,* photopatterning of conductive polymers to strengthen interconnected networks such as the gap junction networks linking PV cells in the mammalian brain.

Short summary

The emerging field of genetically targeted chemical assembly (GTCA) uses cell-specific genetic information to instruct chemical synthesis *in situ*. This Perspective discusses recent progress in GTCA, and outline opportunities that may expand the GTCA toolbox.