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Semiconducting Polymer Nanobioconjugates as Targeted Photothermal Nanomodulator for Remote Activation of Neurons

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Supporting Information Placeholder

ABSTRACT: Optogenetics provides a powerful means for precise control of neuronal activities; However, the requirement of transgenesis and the incapability to extend light response into the deep-tissue-penetrating near-infrared (NIR) range partially limit its application. We herein report an alternative approach to optogenetics using semiconducting polymer nanobioconjugates (SPNs_{bc}) as a photothermal nanomodulator for activation of the thermosensitive ion channels of neurons. SPNs_{bc} are designed to efficiently absorb the NIR light at 808 nm and have a photothermal conversion efficiency that is 1.15-fold higher than that of gold nanorods. By virtue of the fast heating capability in conjunction with the precise targeting to the thermosensitive ion channel, SPNs_{bc} can specifically and rapidly activate the intracellular Ca²⁺ influx of neuronal cells in a reversible and safe manner. Our study thus provides an organic nanoparticle-based strategy that eliminates the need for genetic transfection to remotely regulate cellular machinery.

Spatial and temporal regulation of cellular activity is imperative to decipher underlying physiological processes and develop novel therapeutic modalities. Optogenetics combines optical methods with molecular genetics, providing a powerful means for precise control of defined events in specific cells of living tissues.¹ In particular, microbial opsins such as channelrhodopsin and halorhodopsin are selectively expressed in the targeted neuronal cells, allowing light to control the relevant ion channel and subsequently activate neuronal activity.² With the advantage of simultaneous input-output interrogation of neurons that is not accessible for electrical stimulation, optogenetics has opened new landscapes for neuroscience.³ However, because microbial opsins and their variants can only absorb the light ranging from ~470 to 630 nm,⁴ current optogenetics methods are limited to the visible light spectrum that has a shallow depth of tissue penetration.⁵ Moreover, the requirement for transgenesis complicates the implementation of optogenetics and partially constrains its application.

One emerging alternative to optogenetics is the nanoparticle-based direct thermal stimulation of unmodified neurons.^{6,7,8,9,10,11} Magnetic iron oxide nanoparticles and optical metallic nanoparticles can respectively convert low-

radiofrequency alternating magnetic fields and photon energy into heat,^{12,13} giving rise to increased local temperature. Such a nanoparticle-mediated temperature change can be transduced by a thermosensitive protein channel into cellular responses. However, probably due to the slow magnetothermal generation, magnetic iron oxide nanoparticles required tens to thousands of seconds to induce increased calcium ion (Ca²⁺) influx, which exceeded temporal dynamics of neuronal firing. In comparison, photothermal stimulation based on gold nanoparticles or carbon nanotubes showed relatively shorter activation time (~30 s). However, current methods primarily relied on the electrostatic or hydrophobic interactions between metallic nanoparticles and plasma membrane, which performed nonspecifically and had the risk of damaging cellular membrane and causing cytotoxicity. Thereby, selective and rapid stimulation of specific thermosensitive channels of unmodified neuron remains to be demonstrated.

Figure 1. Synthesis and Characterization of SPNs. (a) Synthetic route of SP2 via Stille polymerization under the reaction conditions (i) PdCl₂(PPh₃)₂ and 2,6-di-*tert*-butylphenol, 100 °C for 12 h. (b) Chemical structures of SP1. (c) Schematic illustration of synthesis of SPNs. (d) Representative TEM image of SPNs: SPN2. (e) Represent DLS profiles of SPNs. (f) Photos of SPN solutions (18 μg mL⁻¹).

Figure 2. Optical and photothermal characterization of SPNs. (a) Absorption of SPNs and GNR. (b) Temperatures of SPN and GNR solutions as a function of laser irradiating time. (c) Thermal images of SPNs and GNR at their respective maximum temperatures. (d) Stability study of SPN2 and GNR under the photothermal heating and natural cooling cycles. The concentrations of nanoparticles were $18 \mu\text{g mL}^{-1}$ in $\times\text{PBS}$ ($\text{pH} = 7.4$). The laser irradiation wavelength was at 808 nm with a power of 1 W cm^{-2} .

In this study, we report the design and synthesis of semi-conducting polymer nanobioconjugates (SPN_{bc}) with high photothermal conversion efficiency and demonstrate their proof-of-concept application in specific photothermal stimulation of neuronal cells by targeting transient receptor potential cation channel subfamily V member 1 (TRPV1). TRPV1 is an ion permeable polymodal channel that can be activated by exogenous and endogenous physical and chemical stimuli such as heat.¹⁴ TRPV1 is widely expressed across the mammalian nervous system and thus serves as an intrinsic thermosensitive channel for us to precisely control the Ca^{2+} influx in neuronal cells. Semiconducting polymer nanoparticles (SPNs) are chosen as the photothermal nanomodulator to remotely increase the local temperature and subsequently activate TRPV1. Because SPNs are transformed from semiconducting polymers (SPs) that are completely organic and biologically inert, they circumvent the issue of heavy metal ion-induced toxicity to living organisms and possess good biocompatibility.^{15,16} In addition to molecular imaging applications,^{17,18,19} we recently revealed that SPNs can convert photon energy into heat,²⁰ permitting sensitive photoacoustic imaging and efficient photothermal therapy of tumors.^{21,22} However, to fulfill their function in photothermal stimulation of neuronal cells, development of SPNs with high photothermal conversion efficiency is essential.

To align the photothermal features of SPNs with the near-infrared (NIR) laser at 808 nm ,²³ a new polymer, poly(cyclopentadithiophene-*alt*-diketopyrrolopyrrole) (SP_2), was designed and synthesized *via* Stille polymerization between 2,6-dibromo-4,4-bis(2-ethylhexyl)cyclopenta[2,1-*b*:3,4-*b'*]dithiophene (1) and 2,5-bis(2-ethylhexyl)-3,6-bis(5-(trimethylstannyl)thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (2). Different from its analog poly(cyclopentadithiophene-*alt*-benzothiadiazole) (SP_1 , Figure 1b), incorporation of diketopyrrolopyrrole into the backbone was used to narrow down the band gap and thus further shift the absorption into the NIR region. SP_1 and SP_2

were respectively transformed into water-soluble nanoparticles *via* nano-coprecipitation with an amphiphilic diblock copolymer, polystyrene-*b*-poly(acrylic acid) (PS-PAA) (Figure 1c). The presence of PS-PAA not only conferred SPNs with good water-solubility but also yielded the carboxyl groups on the nanoparticle surface for post-bioconjugation with TRPV1 antibody. The synthesized SPNs had uniform spherical morphology with the hydrodynamic diameters of $\sim 25 \text{ nm}$ (Figures 1d&1e). The nanoparticle solutions were clear (Figure 1f) and remained stable for months with no obvious change in size (Figure S2, Supporting Information).

The optical and photothermal properties of SPNs were studied and compared with the polyethylene glycol (PEG) coated gold nanorods (GNRs). Due to the enhanced charged transfer, the maximum absorption of SPN_2 was red-shifted by 100 to 766 nm as compared to that of SPN_1 (660 nm) (Figure 2a). GNR had the maximum at 780 nm and thus served as a fair control for SPN_2 . The peak mass extinction coefficient of SPN_2 was $87 \text{ cm}^{-1} \text{ mg}^{-1} \text{ mL}$, which was similar to that of SPN_1 ($90 \text{ cm}^{-1} \text{ mg}^{-1} \text{ mL}$) and 4.35 -fold larger than that of GNR ($20 \text{ cm}^{-1} \text{ mg}^{-1} \text{ mL}$). Under continuous laser irradiation at 808 nm , all the nanoparticle showed gradually increased solution temperature and reached plateau at $t = 360 \text{ s}$ (Figure 2b). At each time point, the temperature of SPN_2 was higher than other nanoparticles, indicating its faster heating capability. The maximum photothermal temperature that SPN_2 could reach is 70°C under the tested experimental conditions, which was ~ 1.4 -fold higher than SPN_1 and GNR (Figure 2c).

The photothermal conversion efficiency of SPN_2 was calculated to be 20.71% , which was 1.15 -fold higher than that of GNR (17.96%) but 1.49 -fold lower than that of SPN_1 (30.83%). Thereby, the order of the maximum photothermal temperatures ($\text{SPN}_2 > \text{SPN}_1 \approx \text{GNR}$) was not solely determined by the order of their photothermal conversion efficiencies ($\text{SPN}_1 > \text{SPN}_2 > \text{GNR}$); rather, it is directly affected by their mass extinction coefficients at 808 nm . The photothermal stability of SPN_2 and GNR was studied by reversibly heating and cooling the nanoparticle solutions (Figure 2d). SPN_2 exhibited slightly increased maximum temperature after the first heating cycle because the solution did not completely cool back to the room temperature before the new heating cycle. Nevertheless, the maximum temperatures of SPN_2 remained nearly the same from cycles 2 to 6. In contrast, GNR exhibited gradually decreased maximum temperature, which was due to their susceptibility to laser-induced deformation.²⁴ Thereby, these data verified that SPN_2 had higher photothermal conversion efficiency, faster heating capability and better photothermal stability as compared with GNR.

To specifically activate the thermosensitive ion channels of neurons, SPNs were conjugated with anti-TRPV1 antibody through a carbodiimide coupling reaction between the carboxyl group of SPNs and the amine group of anti-TRPV1 antibody (Figure 3a). The agarose gel electrophoresis revealed that SPN_{bc} migrated much less as compared to SPN_1 (Figure 3b). Additionally, DLS showed that the resulting SPN_{bc} exhibited increased hydrodynamic diameter from 25 to 37 nm (Figure 3c). These changes implied that anti-TRPV1 antibody was successfully conjugated on the nanoparticle surface.

Because SPN_{bc} was fluorescent in the NIR region with the emission maximum at 838 nm (Figure S3, Supporting Information), the targeting capability of SPN_{bc} was examined using fluorescence cell imaging. Mouse neuroblastoma/rat

dorsal root ganglion (DRG) neuron hybrid ND7/23 cells that intrinsically express TRPV1 on their plasma membrane were chosen as the example neural cells, while human cervical carcinoma HeLa cells were used as the TRPV1 negative control. After incubation with SPN_{1bc} and multiple washing steps to remove the free nanoparticles, live-cell imaging was conducted. Red fluorescence around the cell periphery was detected for ND7/23 cells; in contrast, no fluorescence was visible for HeLa cells under the same experimental conditions. Because TRPV1 receptors were intrinsically expressed on the plasma membrane of DRG neurons, the fluorescence imaging data clearly confirmed that SPN_{1bc} effectively bound to the TRPV1 ion channel. This ensured that the localized heat from the SPN_{bc} nanomodule could be quickly dissipated to the TRPV1 ion channel, suggesting the feasibility of specific photothermal stimulation of neuronal activity.

Figure 3. Synthesis and characterization of SPNs_{bc}. (a) Synthesis of SPNs_{bc} via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/N-hydroxysuccinimide (NHS) coupling reaction. (b) Hydrodynamic diameters and (c) agarose gel electrophoresis of SPN1 and SPN_{1bc}. (d) Fluorescence cell images of mouse neuroblastoma/DRG neuron hybrid ND7/23 cells and HeLa cells treated with SPN_{1bc}. Cy5 channel was used to excite the cells and fluorescence signals were detected at 725 ± 50 nm.

The ability of SPNs_{bc} to stimulate TRPV1 was evaluated using mouse neuroblastoma/rat DRG neuron hybrid ND7/23 cells as the TRPV1-positive cells. HeLa cells were used as the TRPV1-negative control cells. The Ca^{2+} influx, as an important biological processes that plays a vital role in neurotransmitter release,²⁵ was monitored in real-time using a commercially available fluorescent turn-on intracellular indicator (Fluo-8) (Figure 4a). Without nanoparticle treatment, no fluorescence intensity increase of Fluo-8 was observed for ND7/23 cells after irradiation at 808 nm for 3 s, proving that the laser irradiation alone was not able to activate the TRPV1 ion channel under our experimental conditions. In contrast, a significant increase in the fluorescence intensity of Fluo-8 was observed for SPN_{2bc}-treated but not for SPN_{1bc}-treated ND7/23 cells (Figure 4b). This should be ascribed to the faster heating capability of SPN_{2bc} relative to SPN_{1bc} as observed in solution (Figure 2b). Accordingly, SPN_{2bc} was able to quickly increase the local temperature of TRPV1 above the threshold (43°C) to activate the TRPV1 ion channel and induce the intracellular Ca^{2+} influx; this was not possible for SPN_{1bc} even with the longer laser irradiation time. Such a SPN-dependent activation highlighted the importance of molecular design of SP for photothermal applications. The fact that SPN_{2bc} could not induce the change in the fluorescence of TRPV1-negative HeLa cells further confirmed that the observed fluorescence enhancement resulted from the photothermal activation of TRPV1 ion channels. These find-

ings reflected that the cellular membrane of HeLa cells remained intact under the laser irradiation conditions; otherwise, membrane disruption could lead to the nonspecific Ca^{2+} influx and in turn increased fluorescence.²⁶ In fact, the cell viability assays verified that SPNs have good cytocompatibility and there is no cytotoxicity induced by such a short laser irradiation process (Figures S4 and S5, Supporting Information). The safety of SPN_{2bc}-mediated photothermal stimulation of neuronal cells should benefit from the efficient photothermal conversion of SPN₂ that minimizes the laser irradiation time and in turn reduce the probability of noxious heat.

Figure 4. NIR Photothermal stimulation of mouse neuroblastoma/DRG neuron hybrid ND7/23 cells. (a) Schematic illustration of SPN_{bc} controlled photothermal activation of Ca^{2+} channels in neurons. The intracellular concentration of Ca^{2+} was monitored in real-time by using Fluo-8 as the indicator, which turned on its fluorescence upon binding with Ca^{2+} . (b) Fluorescence images of ND7/23 or HeLa treated with SPN_{1bc} or SPN_{2bc} before and after laser irradiation time at 808 nm ($104 \mu\text{W} \mu\text{m}^{-2}$) for 2 s. (c) The fluorescence intensity of Fluo-8 as a function of laser irradiation time. (d) Changes in the fluorescence intensity of Fluo-8 with the laser irradiation at 808 nm switching on and off at an interval of 0.5 s.

To determine whether SPN_{bc} had the capability of simultaneous input-output interrogation of neuronal cells, monitoring of Fluo-8 fluorescence was conducted in real time along with photothermal stimulation by SPNs_{bc}. As soon as the laser irradiation initiated, Fluo-8 fluorescence immediately increased until reaching its plateau at ~ 1.5 s for SPN_{2bc}-treated ND7/23 cells (Figure 4c); this was not observed for both SPN_{1bc}-treated and untreated cells. Due to the limited temporal resolution of the fluorescence microscopy used, the

first data point was collected at 100 ms after laser irradiation. At this time point, the fluorescence intensity of Fluo-8 already increased by 2-fold as compared to that before laser irradiation, implying the instant occurrence of intracellular Ca^{2+} influx upon laser irradiation. This phenomenon verified that the SPN_{bc} -based photothermal approach could activate neurons within milliseconds. In addition, the TRPV-1 Ca^{2+} channel could be reversibly activated and silenced by switching the laser irradiation on and off at an interval of 0.5 s (Figure 4d and Figure S6, Supporting Information). These data not only demonstrated that the capability of simultaneous input-output interrogation of neuronal cells within microseconds but also highlight the rapid photothermal stimulation of neuronal cells as a result of rapid heating capability of SPN_2 .

In conclusion, we have designed and synthesized NIR-absorbing organic SPNs with the higher photothermal conversion efficiency, faster heating capability and better photothermal stability as compared with GNR and demonstrated their application in the specific thermal stimulation of neuronal cells without genetic transfection of microbial opsins. The surface amenability of SPNs enabled efficient conjugation with anti-TRPV1 antibody, leading to a smart photothermal bioconjugates (SPNs_{bc}) that precisely targeted the thermosensitive TRPV1 ion channel on the plasma membrane of neuronal cells. The high photothermal performance and the targeting capability minimized the simulation time and avoided the off-target side effect, respectively, both of which contributed to reduced probability of noxious heat to living cells. Upon transient NIR laser irradiation at 808 nm, SPN_{bc} acted as a wireless remote nanomodulator to rapidly and specifically activate the TRPV1 ion channel and induce the intracellular Ca^{2+} influx within milliseconds in a safe and reversible manner.

To the best of our knowledge, our study provides the first example of an organic nanoparticle based photothermal system for precise control of defined events in specific cells of living tissues; this system could represent a potential alternative to optogenetics. In addition to providing insights into fundamental neuroscience, the structural flexibility of SPNs will facilitate the development of novel therapeutic approaches such as photothermic regulation of gene therapy and photothermal therapy of astrocytomas that has intrinsically expressed TRPV1.²⁷

ASSOCIATED CONTENT

Supporting Information

Detailed experiment procedures and supporting figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interests.

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REFERENCES

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- 1 K Deisseroth, *nature methods*, 2011, 8, 26-29.
 - 2 Optogenetic control of intracellular signaling pathways. K. Zhang, B. Cui. *Trends in Biotechnology*, 2015, 33, 92-100.
 - 3 Fenno, L.; Yizhar, O.; Deisseroth, K. The Development and Application of Optogenetics. *Annu. Rev. Neurosci.* 2011, 34, 389–412.
 - 4 Lin, J. Y.; Knutsen, P. M.; Muller, A.; Kleinfeld, D.; Tsien, R. Y. ReaChR: A Red-Shifted Variant of Channelrhodopsin Enables Deep Transcranial Optogenetic Excitation. *Nat. Neurosci.* 2013, 16, 1499–1508.
 - 5 Dye-Sensitized Core/Active Shell Upconversion Nanoparticles for Optogenetics and Bioimaging Applications. *ACS Nano*, 2016, 10, 1060–1066
 - 6 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.* 2010, 5, 602–606.
 - 7 Sarah A. Stanley, Jennifer E. Gagner, Shadi Damanpour, Mitsukuni Yoshida, Jonathan S. Dordick, Jeffrey M. Friedman, *Science*, 2012, 336, 604-608.
 - 8 Ritchie Chen, Gabriela Romero, Michael G. Christiansen, Alan Mohr, Polina Anikeeva, Wireless magnetothermal deep brain stimulation. *Science*, 2015, 347, 1477-1480.
 - 9 Hirotaka Nakatsuji, Tomohiro Numata, Nobuhiro Morone, Shuji Kaneko, Yasuo Mori, Hiroshi Imahori, and Tatsuya Murakami, *Angew. Chem. Int. Ed.* 2015, 54, 11725 –11729.
 - 10 Eijiro Miyako et al., *Angew. Chem. Int. Ed.* 2014, 53, 13121 –13125.
 - 11 *ACS Nano*, 2016, 10, 3881–3885.
 - 12 J. Yu, C. Yang, J. Li, Y. Ding, L. Zhang, M. Z. Yousaf, J. Li., R. Pang, L. Wei, L. Xu, F. Sheng, C. Li, G. Li, L. Zhao, and Y. Hou, Multifunctional Fe₅C₂ Nanoparticles: a Targeted Theranostic Platform for Magnetic Resonance Imaging and Photoacoustic Tomography-Guided Photothermal Therapy, *Adv Mater*, 2014, 26, 4114-4120.
 - 13 Kai Cheng, Sri-Rajasekhar Kothapalli, Hongguang Liu, Ai Leen Koh, Jesse V. Jokerst, Han Jiang, Meng Yang, Jinbo Li, Jelena Levi, Joseph C. Wu, Sanjiv S. Gambhir, and Zhen Cheng, "Construction and Validation of Nano Gold Tripods for Molecular Imaging of Living Subjects," *J. Am. Chem. Soc.* 136, 3260-3571. 2014.
 - 14 M. Tominaga, M. J. Caterina, A. B. Malmberg, T. A. Rosen, H. Gilbert, K. Skinner, B. E. Raumann, A. I. Basbaum, D. Julius, *Neuron* 1998, 21, 531 –543.
 - 15 Changfeng Wu, D. T. Chiu. *Angew. Chem. Int. Ed.* 2013, 52, 3086 – 3109
 - 16 C. L. Zhu, L. B. Liu, Q. Yang, F. T. Lv, S. Wang, *Chem. Rev.* 2012, 112, 4687.
 - 17 K. Pu, A. J. Shuhendler, J. H. Rao, *Angew. Chem. Int. Ed.* 2013, 52, 10325.
 - 18 A. J. Shuhendler, K. Pu, L. Cui, J. P. Uetrecht, J. H. Rao, *Nat. Biotechnol.* 2014, 32, 373.
 - 19 Wu, C. F.; Schneider, T.; Zeigler, M.; Yu, J. B.; Schiro, P. G.; Burnham, D. R.; McNeill, J. D.; Chiu, D. T. Bioconjugation of Ultrabright Semiconducting Polymer Dots for Specific Cellular Targeting. *J. Am. Chem. Soc.* 2010, 132, 15410–15417.

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- 20 Pu K, Shuhendler AJ, Jokerst JV, Mei J, Gambhir SS, Bao Z, Rao J. Semiconducting polymer nanoparticles as photoacoustic molecular imaging probes in living mice. *Nature Nanotech.*, 2014, 9, 233-239
- 21 Yan Lyu, Yuan Fang, Qingqing Miao, Xu Zhen, Dan Ding, Kanyi Pu. Intraparticle molecular orbital engineering of semiconducting polymer nanoparticles as amplified theranostics for in vivo photoacoustic imaging and photothermal therapy. *ACS Nano*, 2016, 10, 4472–4481.
- 22 Qingqing Miao, Yan Lyu, Dan Ding, Kanyi Pu. Semiconducting oligomer nanoparticles as an activatable photoacoustic probe with amplified brightness for in vivo imaging of pH. *Advanced Materials*, 2016, 28, 3662–3668
- 23 *Nat Med.* 2011 Dec; 17(12): 1685–1691.
- 24 Link, S., Burda, C., Nikoobakht, B. & El-Sayed, M. A. Laser-induced shape changes of colloidal gold nanorods using femtosecond and nanosecond laser pulses. *J. Phys. Chem. B* 104, 6152–6163 (2000).
- 25 B. Alberts, D. Bray, K. Hopkin, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Essential Cell Biology*, 3rd ed., Garland, New York, 2009.
- 26 J. Baumgart, L. Humbert, E. Boulais, R. Lachaine, J. J. Lebrun, M. Meunier, *Biomaterials* 2012, 33, 2345–2350.
- 27 Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1. *Nature Medicine*, 2012, 18, 1232.