Electronic Supplementary Material

Optical control of neuronal activities with photoswitchable nanovesicles

Hejian Xiong[1], Kevin A. Alberto[2], Jonghae Youn[1], Jaume Taura[3], Johannes Morstein[4], Xiuying Li[1], Yang Wang[1], Dirk Trauner[4], Paul A. Slesinger[3] (✉), Steven O. Nielsen[2] (✉), and Zhenpeng Qin[1,5,6,7] (✉)

[1] *Department of Mechanical Engineering, The University of Texas at Dallas, Richardson, TX 75080, USA*
[2] *Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080, USA*
[3] *Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA*
[4] *Department of Chemistry, New York University, New York, NY 10012, USA*
[5] *Department of Bioengineering, The University of Texas at Dallas, Richardson, TX 75080, USA*
[6] *Department of Surgery, The University of Texas at Southwestern Medical Center, Dallas, TX 75080, USA*
[7] *Center for Advanced Pain Studies, The University of Texas at Dallas, Richardson, TX 75080, USA*

Supporting information to https://doi.org/10.1007/s12274-022-4853-x

Address correspondence to Paul A. Slesinger, paul.slesinger@mssm.edu; Steven O. Nielsen, steven.nielsen@utdallas.edu; Zhenpeng Qin, zhenpeng.qin@utdallas.edu

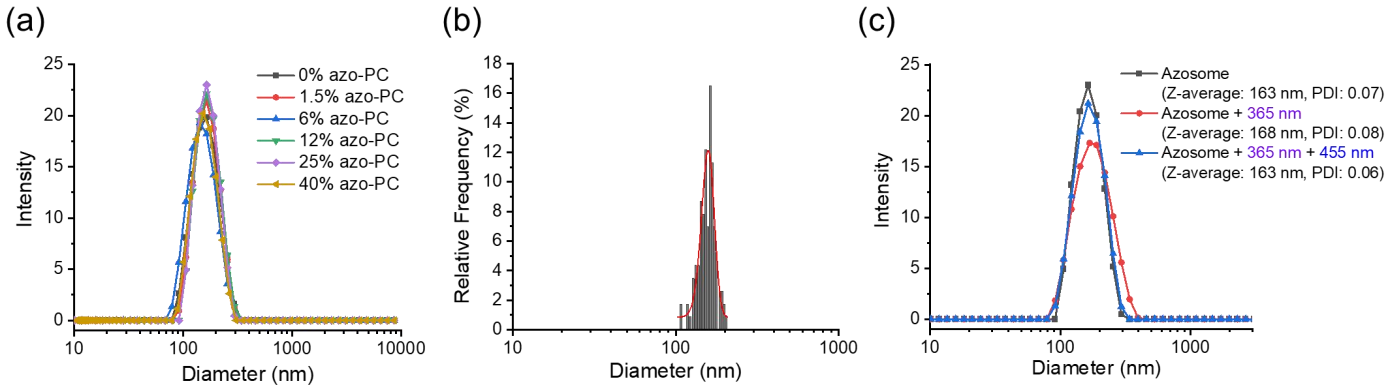

Figure S1 (a) The size distribution of azosomes with different percentages of azo-PC. (b) Size distribution of azosomes measured by Cryo-TEM (n=100 counts). Red line refers to the Gaussian fit. (c) The change of size distribution of azosomes before and after the 365 and 455 nm light irradiation (azo-PC: 25%, 30 s, 25 mW/cm^2).

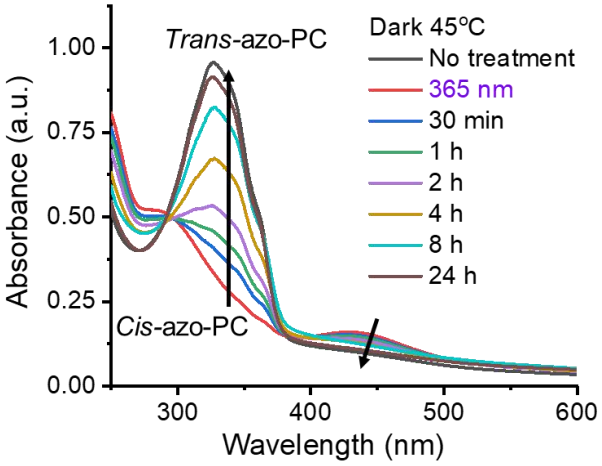

Figure S2 UV-Vis spectra change of azosomes in the dark at 45°C. 365 nm light irradiation (25 mW/cm^2, 60 s) was delivered to azosomes to switch *trans*-azo-PC to *cis*-azo-PC at 0 min.

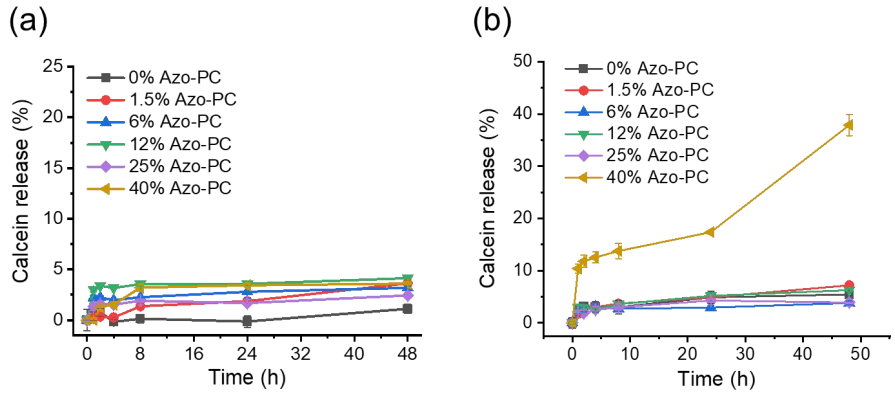

Figure S3 Plots of calcein release over time. The calcein leakage from azosomes shown with different percentages of azo-PC at two different temperatures (a) 22°C and (b) 37 °C in 0.01 M PBS. Data are expressed as Mean ± S.D.

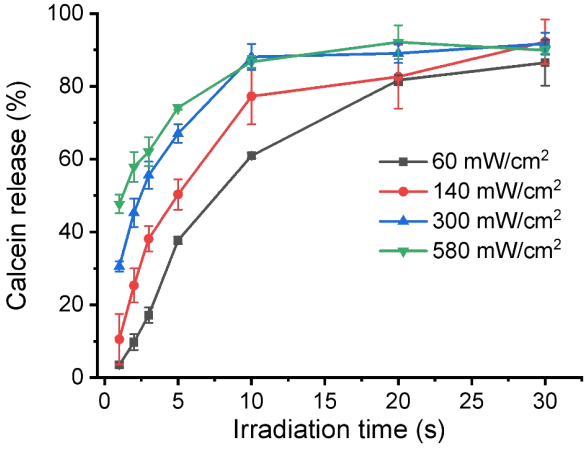

Figure S4 Calcein release efficiency plotted as a function of irradiation time at different light (405 nm) intensities. Azo-PC: 12%. Data are expressed as Mean ± S.D.

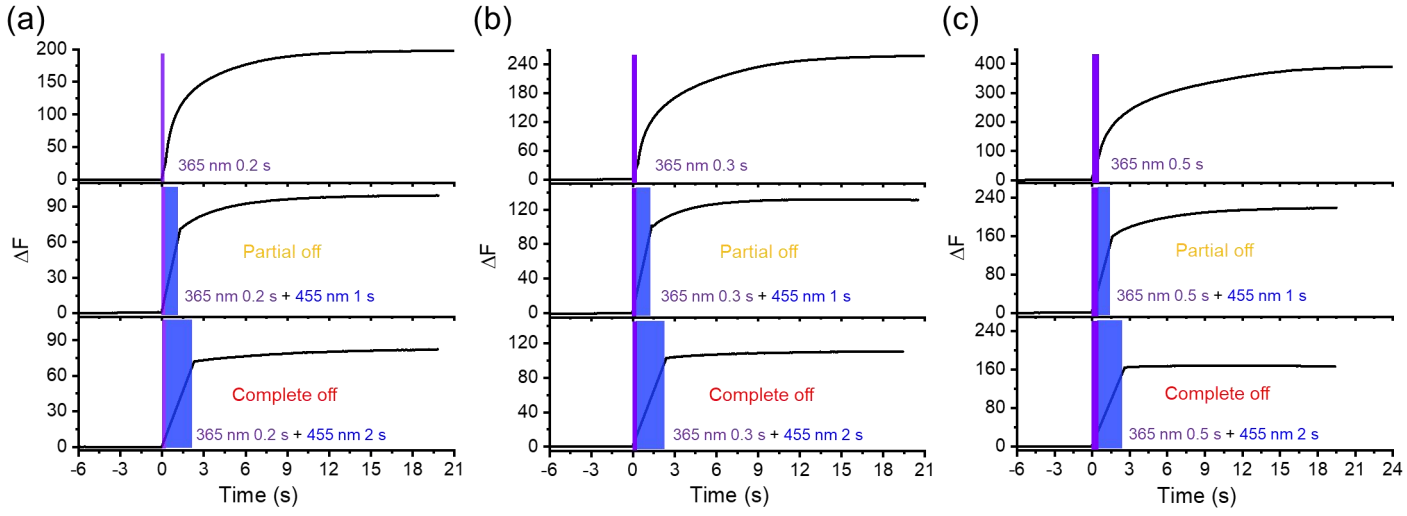

Figure S5 Real-time fluorescence intensity of calcein-azosome (ΔF) plotted over time under the single irradiation of 365 nm and 455 nm light (40 mW/cm^2). The durations of 365 nm irradiation were 0.2 s, 0.3 s and 0.5 s in (a), (b) and (c), respectively.

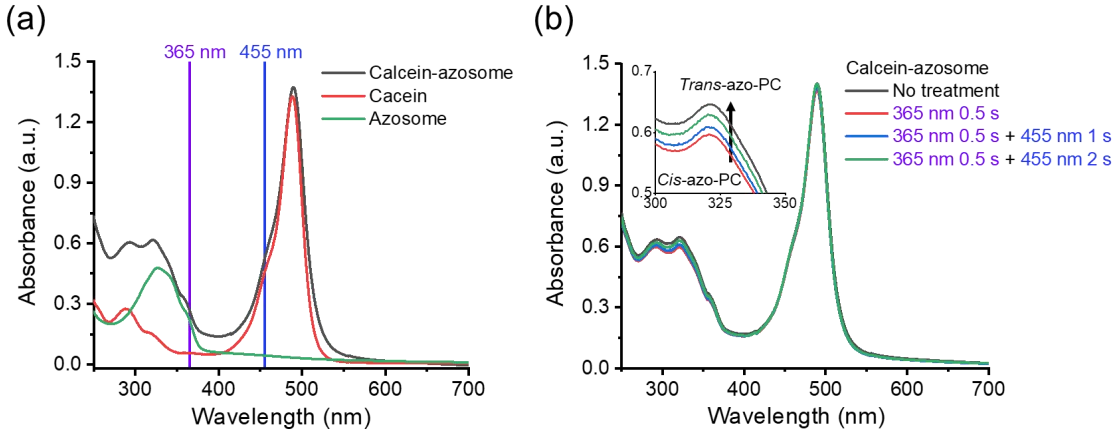

Figure S6 (a) UV-Vis spectra of calcein-azosome, calcein and azosome. (b) UV-Vis spectra of calcein-azosome under different treatments. Sequential irradiation of 365 nm and 455 nm light (40 mW/cm^2) was performed.

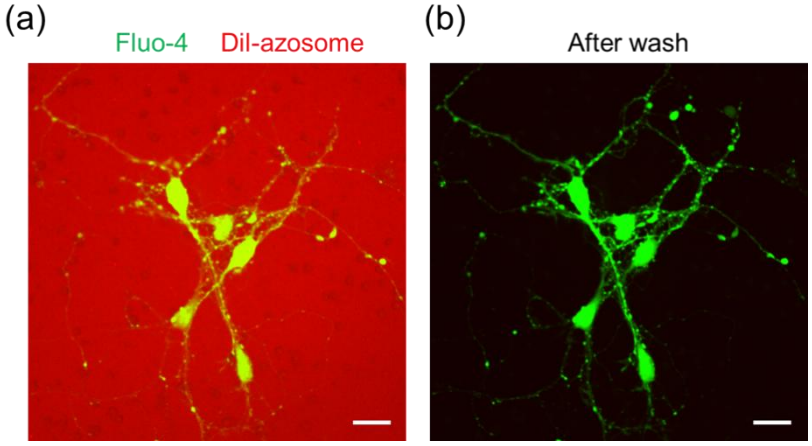

Figure S7 (a)Confocal images of live mouse primary neurons incubated with Dil-azosome (red) for 1 h. Neurons were labelled by fluo-4 (green). The concentration of azosome was 10-fold higher than that in the neuromodulation experiments. (b) Confocal images of live mouse primary neurons after wash. Little or no fluorescence of Dil-azosomes (red) was detected after wash, suggesting that azosomes were not endocytosed by the neurons during the incubation. Scale bar: 20 μm.

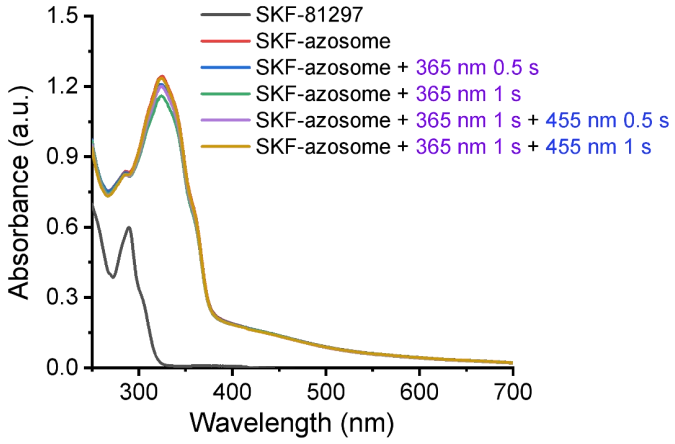

Figure S8 UV-Vis spectra change of SKF-azosome under different treatments (25 mW/cm^2 for 365 nm and 455 nm light).

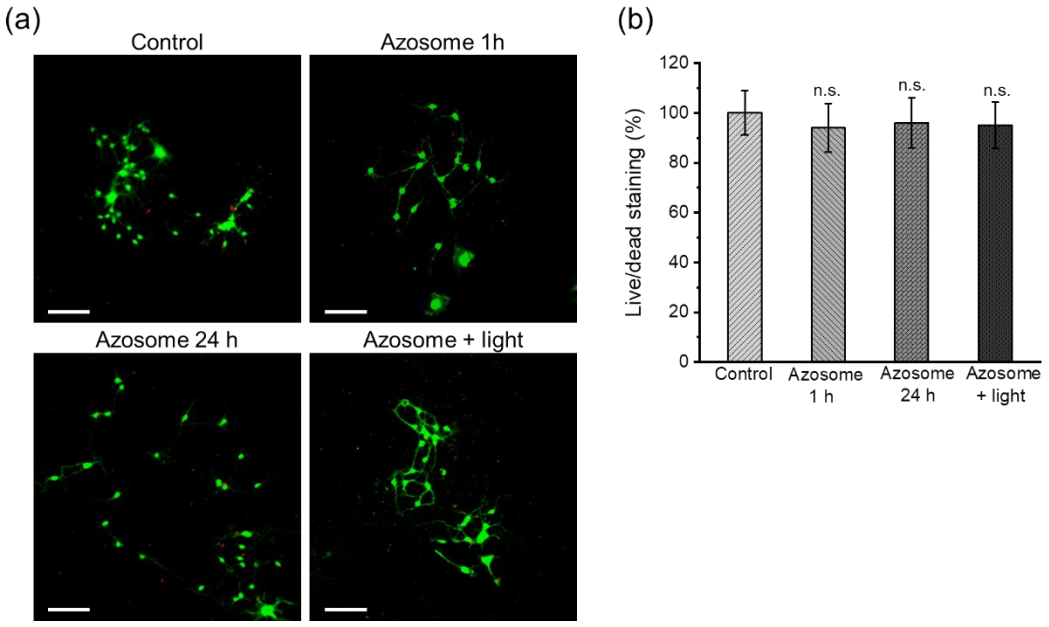

Figure S9 *In vitro* cytotoxicity of azosome. (a) Confocal images of mouse primary neurons stained by calcein (green) and PI (red). Scale bar: 100 μm. (b) The fluorescence intensity ratio of calcein and PI measured with a plate reader after different treatments (light: 365 nm 2s + 455 nm 2 s, 40 mW/cm^2). No significant difference was found between the control group and azosome-treated groups by two-sample Student's t-test.