Biocompatible photoinduced CuAAC using sodium pyruvate


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Materials and Methods

Materials

All chemicals and solvents were purchased from Sigma Aldrich, unless otherwise specified. Phosphoramidites and reagents for the solid-phase DNA synthesis were purchased from Glen Research and ChemGenes. The 5’-hexynyl modifier phosphoramidite was purchased from Glen Research. Q570 CPG Synthesis Column was purchased from Biosearch Technologies. Cyanine 5 (Cy5)-azide was purchased from Lumiprobe. For the solid-phase DNA synthesis, Mermade 4 (Bioautomation) was used following the standard protocols suggested by the manufacturer.

General procedure for SP-CuAAC reaction

SP-CuAAC between Q570 labeled DNA alkyne (0.1 mM) and Cy5-azide (0.11 mM) was conducted by mixing 0.25 mM CuSO$_4$, 1.25 mM tris-hydroxypropyltriazolymethylamine (THPTA) and 100 mM sodium pyruvate in PBS with 3.7% DMSO, to increase the solubility of hydrophobic substrates (i.e., Q570 and Cy5 in this study), without degassing (100 μL final volume). The reactions were performed in Eppendorf tube (500 μL) for 45-180 min under the irradiation of UV light (λ = 365 nm, 2 or 6 mW/cm$^2$). After each reaction, the product was immediately diluted with water and the fluorescence of Q570 and Cy5 dyes were analyzed by microplate reader (The Infinite® M1000, Tecan). The fluorescence of Q570 was analyzed by measuring the emission of the dye at 568 nm with excitation at 548 nm. Fluorescence energy transfer from Q570 to Cy5 was performed by exciting the donor Q570 at 548 nm and measuring the emission of Cy5 at 670 nm.

Electrochemical analysis of SP-CuAAC system

The electrochemical characterizations were performed in a 7-neck electrochemical cell, equipped with a 3-electrode system and connected to an Autolab PGSTAT302N potentiostat/galvanostat (Metrohm) run by a PC with NOVA 2.0 software. The 3-electrode system was composed by: i) a Pt foil counter electrode; ii) a saturated calomel electrode (SCE) as reference electrode; iii) a glassy carbon (GC) disk tip (3 mm dia., Metrohm), connected to a rotating disk electrode (RDE) system, as working electrode. Before each experiment, the GC disk was cleaned by polishing with a 0.25 microns diamond paste, followed by ultrasonic rinsing in ethanol for 5 min. The cell was surrounded by an UV lamp and aluminum foil was employed to avoid light dispersion. The analysis was performed under inert atmosphere (nitrogen).

Time course SP-CuAAC reaction

The SP-CuAAC reaction was conducted following the general SP-CuAAC procedure with 0.1 mM of Q570-labeled DNA alkyne and 0.11 mM Cy5-azide. The reaction was set to run for different time courses without degassing under the UV light (λ = 365 nm, 2 or 6 mW/cm$^2$). The reaction mixture was immediately diluted with water and analyzed by Waters alliance 2690 HPLC (Waters) and the microplate
reader. The conversion was measured from the area of the free DNA peak and DNA-Cy5 conjugates peak at 260 nm absorbance in the HPLC profile.

**Temporal control experiment**

250 μL of the reagent mixture were added to a 96 well plate and the SP-CuAAC reaction was conducted following the general SP-CuAAC procedure, with 0.1 mM of Q570-labeled DNA-alkyne and 0.11 mM Cy5-azide. The reaction was set to run without deoxygenation under the UV light (λ = 365 nm, 6 mW/cm²). Every 15 min of reaction, the UV light was turned on or off and an aliquot of solution was taken to measure the fluorescence of Q570 (λ<sub>ex</sub> = 548 nm, λ<sub>em</sub> = 568 nm) with a microplate reader. The conversion was calculated by substituting the fluorescence decrease factor (F/F0) of Q570 into the linear regression equation from Fig. S5A.

**Calculation of conversion from fluorescence measurement**

For the calculation of F/F0, the fluorescence intensity of Q570 after the reaction (F) was divided by the initial fluorescence intensity of Q570 dye at t = 0 (F0). The measured conversion (HPLC conversion) from Fig. S4a and the corresponding F/F0 were plotted (Fig. S5A) followed by linear regression giving the equation y = -155.1*x + 114.6.

**Cell Culture**

Murine embryonic fibroblasts cell line (NIH3T3; ATCC®CRL-1658™) was grown and maintained in Dulbecco’s Modified Eagle media (DMEM; Gibco) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% Penicillin-Streptomycin (Gibco).

**Cell viability and proliferation assay**

Cytotoxicity was assessed using direct CyQUANT® nucleic acid-sensitive fluorescence assay (Thermo Fisher Scientific) according to the manufacturer’s instructions. Briefly, 25 × 10<sup>3</sup> cells/well were plated in a 96-well microplate (Corning Inc., Corning) and allowed to adhere overnight. 250 μL of the SP-CuAAC reagent cocktails were added to the cells and the reaction was performed in a cold room (4 – 6 ºC) for 30 min to avoid heating of cells. After the reaction, the cells were washed five times with PBS and incubated in cell culture media in a humidified incubator (5% CO<sub>2</sub>, 37ºC). At designated time-points, cells were labeled with CyQUANT® Direct and fluorescence intensities (λ<sub>ex</sub> = 508 nm, λ<sub>em</sub> = 530 nm) were measured with a TECAN spectrophotometer reader. Cytotoxicity was assessed by normalizing fluorescence intensities to control group (no treatment) and plotted as percent viability.
Supplementary Figures

**Fig. S1.** Microplate reader measurement of Cy5 fluorescence intensity ($\lambda_{em} = 670$ nm) under the excitation of Q570 ($\lambda_{ex} = 548$ nm). [Q570-DNA]/[N3-Cy5]/[CuSO4]/[THPTA]/[SP]: 1/1.1/2.5/12.5/1000, [Q570-DNA] = 0.1 mM, in PBS with 3.7 % DMSO at r.t., under UV light (6 mW/cm$^2$) for 2 h.
Fig. S2. Stability analysis of Q570 dye under UV light irradiation. 0.1 mM of Q570-labeled DNA-alkyne in PBS was irradiated with UV light (6 mW/cm²) and aliquots were taken after certain time frames.
Fig. S3. Optimization of SP-CuAAC conditions. The concentrations of SP and CuSO₄ were adjusted accordingly, with respect to the proof-of-concept experiment (Fig. 1 and Fig. S1). (A, B) Effect of sodium pyruvate concentration on the SP-CuAAC yield after 2 h of reaction. Fluorescence measurements of Q570 (A); and Cy5 (B), respectively. (C, D) Effect of copper concentration on the SP-CuAAC yield after 2 h of reaction. Fluorescence measurements of Q570 (C); and Cy5 (D), respectively.
Fig. S4. (A) HPLC profiles for the SP-CuAAC products after various reaction times under UV light (6 mW/cm²); and (B) the fluorescence intensity of the products. (C) HPLC profiles for the SP-CuAAC products after various reaction times under UV light (2 mW/cm²); and (D) the fluorescence intensity of the products.
Fig. S5. SP-CuAAC reaction under the different light sources. The fluorescence intensity changes of (A) Q570; and (B) Cy5 before and after 45 min of reaction under the irradiation of UV light (365 nm, 6 mW/cm$^2$), white light (400–700 nm, 6 mW/cm$^2$), and green light (520 nm, 8 mW/cm$^2$), in PBS with 3.7 % DMSO at r.t. in an ambient atmosphere. [Q570-DNA]/[N3-Cy5]/[CuSO4]/[THPTA]/[SP]: 1/1.1/2.5/12.5/1000, [Q570-DNA] = 0.1 mM.
Fig. S6. (A) Linear regression of conversion versus F/F0. HPLC conversion and fluorescence measurements of Q570 from Fig. S4A and S4B were used for plotting the graph. (B) Comparison of actual conversion (measured by HPLC) and approximate conversion (calculated from F/F0 and the equation obtained by linear regression). HPLC conversion and fluorescence measurements of Q570 from Fig. S4C and S4D were used for the calculation.

* F = fluorescence intensity of Q570 after reaction

* F0 = fluorescence intensity of Q570 before reaction
Fig. S7. Temporal control in SP-CuAAC with a light on/off period of 30 min. Reaction conditions: [Q570-DNA]/[N3-Cy5]/[CuSO4]/[THPTA]/[SP]: 1/1.1/2.5/12.5/1000, [Q570-DNA] = 0.1 mM, in PBS with 3.7 % DMSO at r.t., under UV light (6 mW/cm²) in an ambient atmosphere. The conversion was calculated using the F/F0 of Q570 and the linear regression equation from Fig. S5A.
Fig. S8. Pseudo-first order kinetic of photoreduction of 0.5 mM CuSO$_4$/THPTA + 100mM SP, in water + 0.1 M PBS under UV irradiation, obtaining $k_{\text{reduction}} = 1.9 \times 10^{-3}$ s$^{-1}$. 
**Fig. S9.** UPLC analysis of the SP-CuAAC product in the presence of HEK293 cells (water as eluent). The HEK293 cells were immobilized on the 96-well plate followed by the treatment of SP-CuAAC reagents under the irradiation of 6 mW/cm² UV light for 30 min.