Automated Control of Photoinitiated RAFT Polymerizations in Well Plates

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Statement of Purpose: Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization allows for control of polymer molecular weight and dispersity, and has a wide range of biomedical applications including polymer-drug conjugates, tissue engineering hydrogels, and excipients for drug delivery. RAFT polymerizations can be initiated using a light source including Photo-induced Electron/energy Transfer-RAFT (PET-RAFT). Further, the development of oxygen-tolerant PET-RAFT now enables these reactions to be done in a 96-well plate format, thus providing high-throughput synthesis of combinatorial polymer libraries [1,2]. In addition, polymer conversion has been shown to correlate with the fluorescence of each sample and allows for online monitoring of reaction kinetics [3]. Since reaction kinetics should ideally be maintained for each sample individually, a method that provides both temporal and spatial control of each reaction would allow for robust parallel synthesis of combinatorial polymer libraries. To control each reaction individually, we developed a multiplexed 96-LED matrix controlled by an Arduino and Python capable of adjusting light intensity at each well. In addition, we utilized this lightbox in tandem with robotics and fluorescence tracking to inform our system's utility in driving PET-RAFT reactions across a 96-well plate **(Figure 1)**.

Methods: A surface mount device printed circuit board containing an 8x12 LED dot matrix was created and connected to a secondary circuit consisting of two 74HC595 shift registers, a UDN2981A source driver, and an Arduino. Fluorescence data was collected using a Molecular Devices SpectraMax spectrophotometer, and was exported to Python for analysis. Specifically, the fluorescence ratio of 632 nm and 615 nm after excitation at 410 nm was calculated to indicate the location and conversion of each reaction. Fluorescence data was then imported to Python in real time and used to calculate the change in fluorescence signal between measurements. A fluorescence ratio difference of less than 0.005 signaled Python to turn off the corresponding LED in the matrix. For the PET-RAFT reaction, wells B3, C9, D5, D11, E8, F6, G2, and H11 of a 96-well plate contained 100 µL of 4-acrylomorpholine (NAM), 20 µL of zinc tetraphenylporphoryin (ZnTPP), 10 µL of CTA, and 70 µL of dimethylsulfoxide (DMSO). The polymerization was monitored at 30-minute timepoints, where a robotic arm completed transfers between the lightbox and the spectrophotometer to cycle between polymerization and data collection, respectively. Once the polymerization was determined to be complete by Python, Gel Permeation Chromatography (GPC) was performed to determine the weight average molecular weight (M_w) and polydispersity (PD) of each sample.

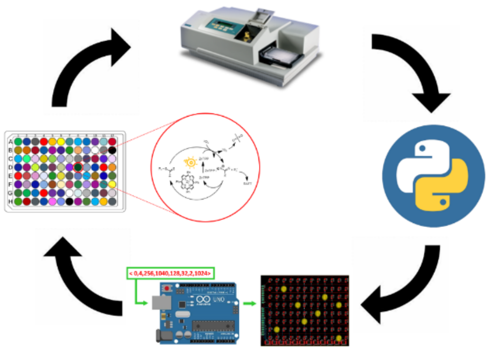

Figure 1. Workflow for automated control of PET-RAFT. Fluorescence is read by the spectrophotometer and sent to Python for analysis. This analyzed file is sent to the Arduino and correlating wells are lit, allowing for spatial and temporal control of PET-RAFT.

Results: Fluorescence tracking performed using the lightbox, robotics, and Python enabled fully automated polymer synthesis. Wells containing a PET-RAFT reaction successfully had their corresponding LEDs turned on and initiated polymerization. The slopes of all fluorescence ratio graphs reached below the .005 cutoff after two hours, which signaled Python to turn the LEDs off. Once Python polymerization was determined to be complete, the plate was collected for GPC analysis. GPC results indicate that all samples reacted uniformly and had similar final molecular weights and polydispersities, where the average M_w was 20769 ± 394 g/mol and the average PD was 1.052 ± .006. Based on the average M_w, polymer conversion was calculated to be about 75%, where the theoretical M_w is 28585 g/mol.

Conclusions: Our custom-designed lightbox demonstrated utility in driving multiplexed PET-RAFT experiments with spatial and temporal control across individual samples. Further development of this platform will enable fully automated synthesis of combinatorial polymer libraries in parallel, allowing high-throughput discovery of copolymers. Furthermore, the addition of machine learning protocols will allow for the directed evolution of polymers with tailored characteristics.

References:

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