

Development of Fluorogenic Atom Transfer Radical Polymerization in Aqueous Media

Christina B. Cooley, Trinity University

Description of Research Progress and Grant Impact

We have made significant progress since May 2017 toward our goals in this proposal of developing a fluorogenic atom transfer radical polymerization (ATRP) reaction in aqueous media. The development of novel approaches to signal amplification in aqueous media could enable new diagnostic platforms for the detection of water-soluble analytes, including biomolecules. Signal amplification by polymerization is a relatively new approach that takes advantage of the growth of a long polymer chain from one initiation event. If that event is coupled to the detection of a desired analyte, the formation of detectable polymer signals analyte presence.

We set out to develop a simple, real-time fluorescence method for signal amplification by *fluorogenic* polymerization, in which non-fluorescent, “dark” monomers become visibly fluorescent upon incorporation into the growing polymer chain. We postulated that a controlled polymerization method would allow for the amount of polymer formed to correspond with initiator or analyte concentration. Atom transfer radical polymerization (ATRP)^{1,2} represents an ideal choice for this purpose as it exhibits controlled, “living” character, robust turnover and kinetics, broad functional group tolerance, bioorthogonal initiation, and ability to react in aqueous solvents, all of which are desirable characteristics for the detection of biological analytes.^{3,4}

Through this grant, we have recently developed the first fluorogenic ATRP reaction as a novel approach for signal amplification (**Figure 1**).⁵ We synthesized non-fluorescent, polyaromatic hydrocarbon probe methacrylamide monomers such as anthracene monomer **2**⁶ (among others including a pyrene and acridine probe backbones) that reveal visible fluorescence when co-polymerized with PEG methacrylate under ATRP conditions (**Figure 1a**). Real-time monitoring of polymer fluorescence tracks with polymerization progress and is observable by quantitative fluorescence detection and qualitatively by eye upon UV light irradiation (**Figure 1b,c**).

We initially optimized the fluorogenic ATRP reaction for detection and published our lab’s first paper in *Chemical Science* in 2019.⁵ We have continued to optimize the reaction for detection by screening alternative surfactants, modifying the catalyst loading and ligand, and designing new fluorogenic monomers that are intrinsically more water soluble by the addition of ionizable groups such as sulfates. These additional improvements will be reported in due course.

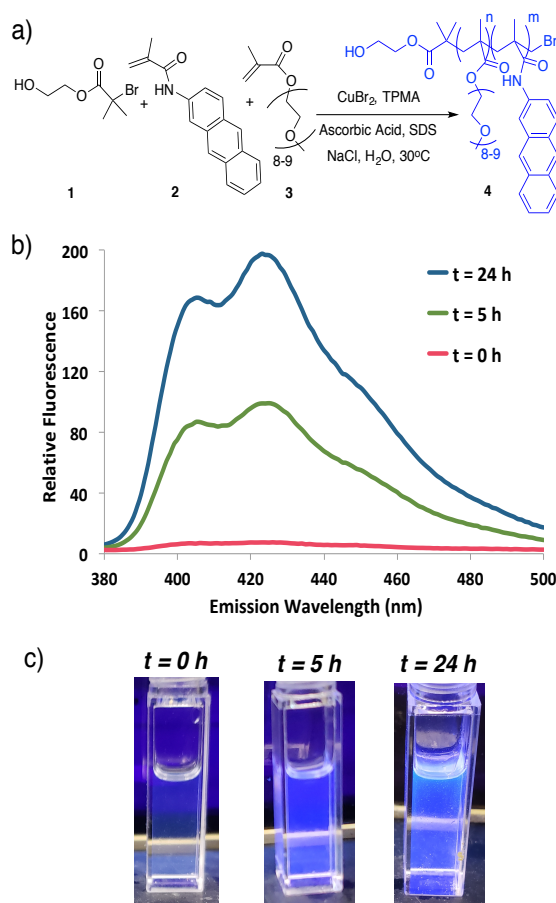


Figure 1. a) Scheme for fluorogenic ATRP reaction with fluorogenic anthracene methacrylamide monomer **2**.

b) Fluorescence emission spectra for reaction in a) at indicated times following 337 nm excitation.

c) Photographs of the reaction at indicated times illuminated by a hand-held UV light (365 nm).

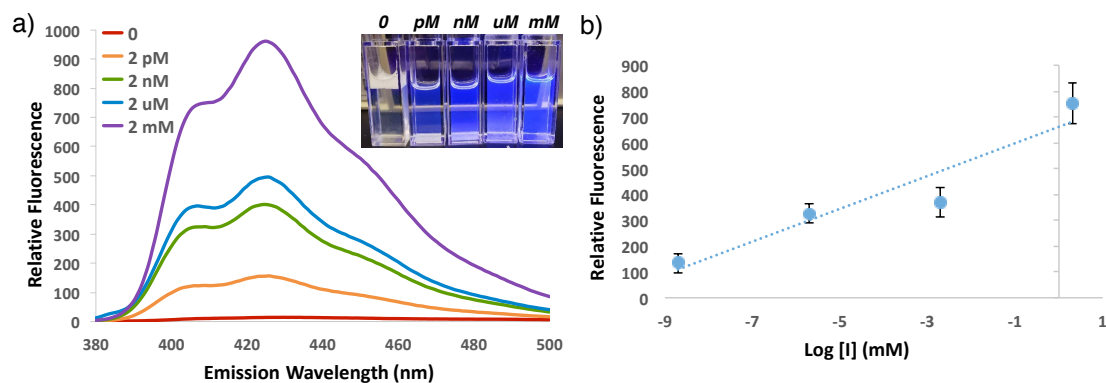


Figure 2. a) Emission spectra and photograph of anthracene ATRP co-polymerization at 30°C with indicated concentrations of initiator **1** at 24 h of reaction time following excitation at 371 nm. b) Plot of relative fluorescence intensity at 426 nm versus initiator concentration from the reaction in (a). Error bars represent standard error of $n=4$ separate trials.

Utilizing water-soluble initiator **1** as a model detectable analyte, we were able to show sensitive detection of initiator down to 2 pM concentration at 24 h of reaction time with our initially optimized conditions (**Figure 2a**). Further, fluorescence tracks with initiator concentration in a relatively linear fashion over a broad concentration range, spanning 9 orders of magnitude (**Figure 2b**). Further optimization by raising the reaction temperature to 60°C allows for the detection of sub-picomolar concentrations of initiator in as little as 1 h of reaction time (**Figure 3**). We further have demonstrated the ability to detect streptavidin protein by fluorogenic ATRP reaction from a designed biotinylated initiator.⁵

Through this American Chemical Society Petroleum Research Fund young investigator grant, we have demonstrated significant progress and developed a new approach toward signal amplification by the design and implementation of a fluorogenic ATRP reaction. The work done through this grant has significantly impacted my personal research career, leading to the lab's first publication (which was highlighted as a paper of the year in *Chemical Science*) and generated results toward multiple follow-up publications which will be reported soon. The first publication has 5 undergraduate student co-authors, including an undergraduate first author, and has significantly impacted the budding research careers of these students. The first author has just started medical school and his research in my lab helped him achieve those career goals. The other students are currently applying for medical school or graduate school positions, and 2 of the 5 are still current undergraduate students that were supported by the PRF grant for summer 2019. All of the current students are still in the laboratory and working to advance our current goals of optimizing the fluorogenic ATRP reaction for detection, the synthesis of intrinsically water soluble fluorogenic monomers, and the development of alternative fluorogenic polymerization platforms including fluorogenic reversible addition-fragmentation chain-transfer (RAFT) and uncontrolled photopolymerization platforms. This grant has been invaluable to getting my independent career off the ground and I plan to further pursue new directions of my research through additional ACS-PRF grant opportunities.

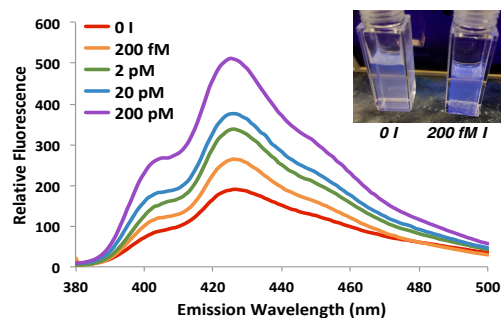


Figure 3. Fluorescence emission spectra for fluorogenic ATRP reaction with various initiator **1** concentrations conducted at 60°C for 1 h following 371 nm excitation.

References

- (1) Wang, J.-S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614–5615.
- (2) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921–2990.
- (3) Matyjaszewski, K. *Macromolecules* **2012**, *45*, 4015–4039.
- (4) Boyer, C. *et al. Chem. Rev.* **2016**, *116* (4), 1803–1949.
- (5) Allen, Z. T.; Sackey-Addo, J. R.; Hopps, M. P.; Tahseen, D.; Anderson, J. T.; Graf, T. A.; Cooley, C. B. *Chem. Sci.* **2019**, *10* (4), 1017–1022.
- (6) Frahn, M. S.; Luthjens, L. H.; Warman, J. M. *Polymer* **2003**, *44* (26), 7933–7938.