Uncontrolled Fluorogenic Radical Polymerization for Biomolecular Detection

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Introduction

- Detection assays require the amplification of discrete molecular interactions into an observable, detectable signal. Radical polymerization reactions inherently amplify a signal.[1,2]
- By coupling polymerization initiator(s) to analytes of interest, we can sensitively detect a given analyte through fluorogenic atom transfer radical polymerization (ATRP).[3] (Figure 1)

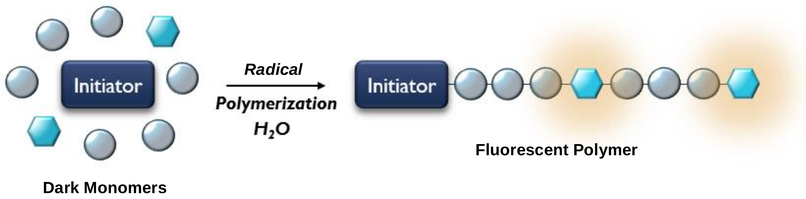
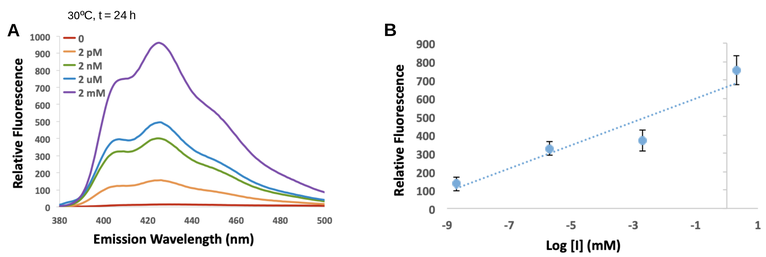


Figure 1. General schematic of fluorogenic radical polymerization.



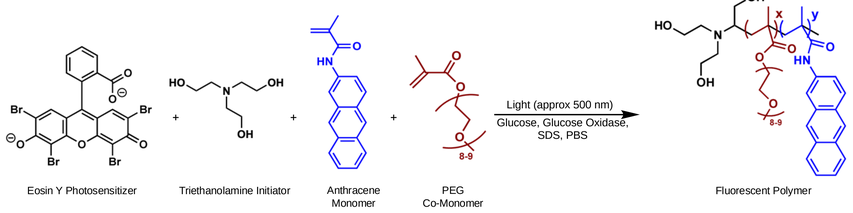
*Excitation at 371 nm

Figure 2. (A) Emission spectra of anthracene ATRP co-polymerization at 30°C with indicated concentrations of initiator 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).[3]

- **Limitations of Original ATRP Approach:**
  - Long reaction times
  - Rigorous degassing required
  - Low throughput

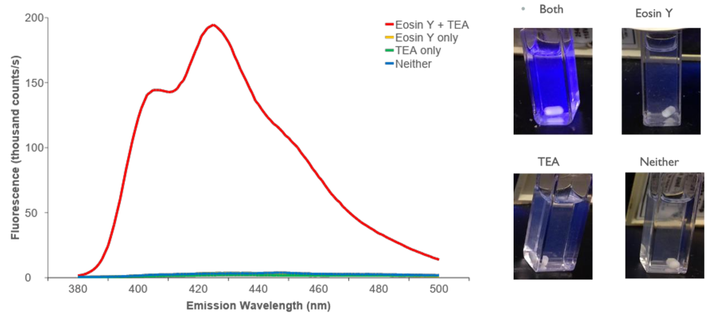
Uncontrolled Fluorogenic Polymerization

Scheme 1. Uncontrolled fluorogenic photoinitiated polymerization.



Dependence on Initiator

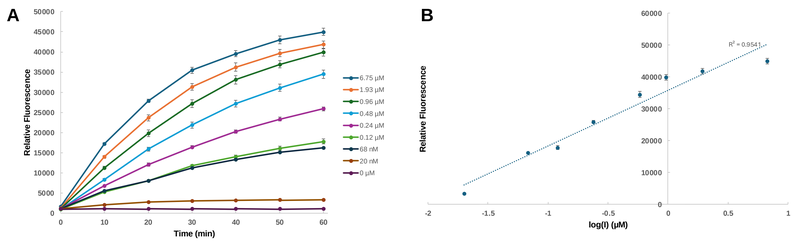
Dependence on Eosin Y and TEA



*t=90 min, 0.35 μM Eosin Y, 150 mM TEA, Glucose Oxidase, Excitation at 371 nm, Experiment performed by Cara Dewitt

Figure 3. General schematic of fluorogenic radical polymerization.

Initiator Concentration Sensitivity

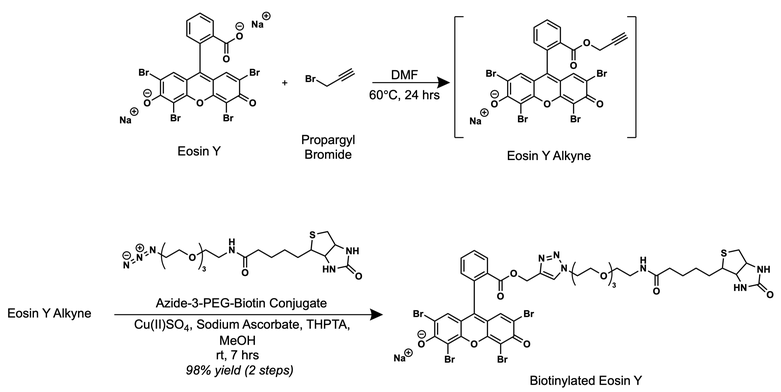


*t=90 min, 10 mM TEA, Glucose Oxidase, Excitation at 371 nm

Figure 4. (A) Detection limit of Eosin Y photosensitizer in an uncontrolled photo polymerization with 10 mM TEA and 371/426 nm ex/em. (B) Plot of relative fluorescence intensity at 426 nm versus initiator concentration from the reaction in (A). Error bars represent standard deviation of n = 3, 4, and 5 separate trials.

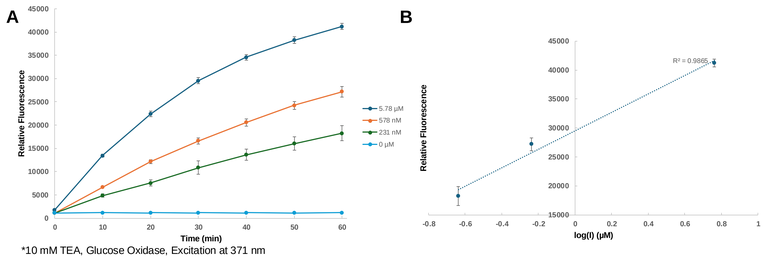
Biotinylated Eosin Y

Scheme 2. Synthesis of biotinylated Eosin Y.



Confirmed by:
^{1}H NMR
LCMS
TLC

Evaluation of Biotinylated Eosin Y

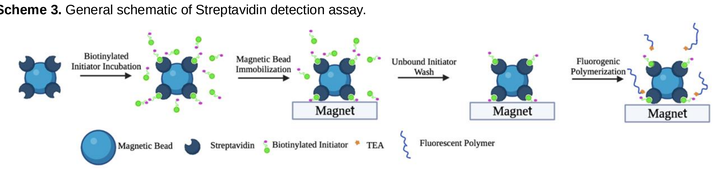


*10 mM TEA, Glucose Oxidase, Excitation at 371 nm

Figure 5. (A) Detection of biotinylated Eosin Y photosensitizer in an uncontrolled photo polymerization with 10 mM TEA and 371/426 nm ex/em. (B) Plot of relative fluorescence intensity at 426 nm versus initiator concentration from the reaction in (A). Error bars represent standard deviation of n = 4 separate trials.

Biomolecular Detection

Scheme 3. General schematic of Streptavidin detection assay.



Detection of Streptavidin-Bound Biotinylated Eosin Y

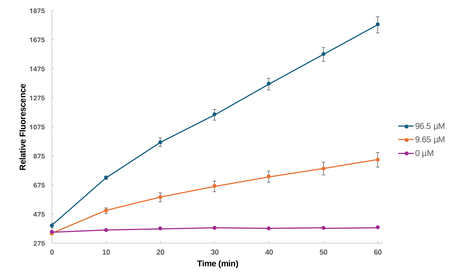


Figure 6. Detection of streptavidin-bound biotinylated Eosin Y photosensitizer in an uncontrolled photo polymerization with 10 mM TEA, 4000 pmol streptavidin/mg, and an 371/426 ex/em. Error bars represent standard deviation of n = 4 separate trials.

*10 mM TEA, 4000 pmol streptavidin/mg, Glucose Oxidase, Excitation at 371 nm

Streptavidin-Bound Biotinylated Eosin Y Fluorescence

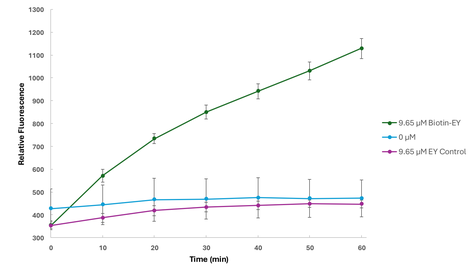


Figure 7. Detection of streptavidin-bound biotinylated Eosin Y photosensitizer and unmodified Eosin Y in an uncontrolled photo polymerization with 10 mM TEA, 4000 pmol streptavidin/mg, and an 371/426 ex/em. Error bars represent standard deviation of n = 4 separate trials.

*10 mM TEA, 4000 pmol streptavidin/mg, Glucose Oxidase, Excitation at 371 nm

Conclusions

- Determination of Eosin Y as model analyte and robust detection limit
- Synthesis of novel biotinylated Eosin Y initiator molecule
- Detection of bound biotinylated Eosin Y over a range of initiator concentrations

References

1. Kaastrup, K.; Sikes, H. D. *Chem. Soc. Rev.* **2016**, *45* (3), 532–545.
2. Qian, H., et al. *Sensors and Actuators B: Chemical* **2010**, *150* (2), 594–600.
3. Allen, Z. T., et al. *Chem. Sci.* **2019**, *10* (4), 1017–1022.

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